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# INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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| (51) International Patent Classification [7] : <br> **C07K 14/00** | **A2** | (11) International Publication Number: **WO 00/46240** <br> (43) International Publication Date: 10 August 2000 (10.08.00) |

(21) International Application Number: PCT/US00/01871

(22) International Filing Date: 27 January 2000 (27.01.00)

(30) Priority Data:
09/244,448        3 February 1999 (03.02.99)        US
09/264,527        8 March 1999 (08.03.99)        US

(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Applications
US                                    09/244,448 (CIP)
Filed on                    3 February 1999 (03.02.99)
US                                    09/264,527 (CIP)
Filed on                    8 March 1999 (08.03.99)

(71) Applicant *(for all designated States except US)*: AMGEN INC. [US/US]; One Amgen Center Drive, Thousand Oaks, CA 91320–1799 (US).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: YOSHINAGA, Steven, Kiyoshi [US/US]; 644 Lynwood Street, Thousand Oaks, CA 91360 (US).

(74) Agents: ODRE, Steven, M. et al.; Amgen, Inc., One Amgen Center Drive, Thousand Oaks, CA 91320–1799 (US).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

**Published**
*Without international search report and to be republished upon receipt of that report.*

(54) Title: NOVEL POLYPEPTIDES INVOLVED IN IMMUNE RESPONSE

(57) Abstract

Novel polypeptides which comprise a receptor–ligand pair involved in T–cell activation are disclosed. Nucleic acid molecules encoding said polypeptides, and vectors and host cells for expressing same are also disclosed. The polypeptides, or agonists and antagonists thereof, are used to treat T–cell mediated disorders.

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# NOVEL POLYPEPTIDES INVOLVED IN IMMUNE RESPONSE

Field of the Invention

5      The present invention relates to polypeptides
that are involved in T-lymphocyte activation.
Specifically, the invention relates to T-lymphocyte
costimulatory polypeptides, the nucleic acids encoding
the polypeptides, expression vectors and host cells for
production of the polypeptides, and compositions and
10     methods for the treatment of diseases related to
immunosuppression and immune activation.

Background of the invention

       For the generation of a proper T-lymphocyte
15     (T-cell) immune response, two signals must be provided
to the T-cell by antigen presenting cells (APC). First,
antigen must be presented to the T-cell receptor (TCR)
via a major histocompatibility complex (MHC), in an
event that determines specificity.  Second, an antigen-
20     independent, costimulatory signal must be delivered by
engagement of members of the B7 family on the APC with
the CD28 protein on T-cells.  A productive immune
response leads to proliferation, differentiation,
clonal expansion, and effect or function. In the
25     absence of the second, costimulatory signal, T-cells
undergo a state of long-lasting antigen-specific
unresponsiveness, termed anergy.
       T-cells initiate the immune response, mediate
antigen-specific effector functions, and regulate the
30     activity of other leukocytes by secreting cytokines.
The T-cell receptor (TCR) distinguishes the T-cell from
other lymphocytes and can bind antigen only when it is
presented by the APC within the context of a MHC.  The
functional activity of a particular T-cell can be
35     correlated with the expression of membrane antigens,
such as CD4 and CD8.  For instance, CD4+ T-cells

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generally function as T helper cells ($T_H$) and are MHC
class II restricted, whereas CD8+ cells generally
function as cytotoxic T-cells ($T_C$) and are MHC class I
restricted.

5        Potent T-cell costimulatory polypeptides
which has been previously identified include
polypeptides termed B7.1 (Freeman et al. J. Immunology
143, 2714-2722 (1989), Freeman et al. Jour. Expt. Med.
174, 625-31 (1991)) and B7.2 (Freeman et al. Science
10   262, 909-911 (1993), and Freeman et al. Jour. Expt.
Med. 178, 2185-2192 (1993)), (or CD80 and CD86,
respectively). These polypeptides are either inducibly
or constitutively expressed on various APCs and are
membrane-bound ligands for CD28 and CTLA-4,
15   respectively, on T-cells. CD28 (Aruffo and Seed Proc.
Natl. Acad. Sci. 84, 8573-8577 (1987) and Gross et al.
J. Immun. 144, 3201-3210 (1990)) is expressed on
resting T-cells and mediates a positive costimulatory
signal. CTLA-4 (Brunet et al. Nature 328, 267-270
20   (1987) and Dariavach et al. Eur. Jour. Immun. 18, 1901-
1905 (1988)) expression is induced upon T-cell
activation and negatively regulates the CD28 signal,
due to its higher binding affinity for B7.1 and B7.2.
Mice without the CTLA-4 gene exhibit dramatically high
25   levels of T-cells, since the switch off mechanism for
the proliferation signal is impaired in the absence of
CTLA-4. This phenotype clearly demonstrates the major
inhibitory effect that the CTLA-4 costimulatory protein
has on T-cell proliferation. Mice lacking CD28 or B7.1
30   or B7.2 have a less severe phenotype, indicating that
alternate pathways for T-cell costimulation may exist.
       There has been considerable interest in the
CD28/CTLA-4 pathway as means for regulating T-cell
activation and proliferation. A chimeric protein
35   containing the extracellular portion of CTLA-4 fused to

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human Fc has strong immunosuppressive effects and has
been studied in a variety of clinical settings.
Antibodies to B7.1 and B7.2 proteins have also been
evaluated for similar indications in the area of
5    immunosuppression.  Anti-CTLA-4 antibodies have shown
utility in promoting T-cell activation.  In addition,
B7.1 and B7.2 gene therapy has shown great promise in
the area of cancer immunotherapy.

        Thus far, CD28, CTLA-4, B7.1 and B7.2 are
10   involved in a single T-cell costimulatory pathway.
Given the capability of modulating an immune response
by regulating T-cell costimulation, it would be
desirable to identify other members of the same or a
separate T-cell costimulatory pathway which may have
15   advantageous properties in regulating host T-cell
function and immune response.

        Accordingly, it is an object of the invention
to provide novel polypeptides for stimulation of T-cell
activity and/or proliferation.  It is a further object
20   of the invention to use the novel polypeptides for the
prevention and treatment of T-cell mediated immune
disorders.


Summary of the Invention
25          Surprisingly, two novel polypeptides of a
T-cell costimulatory pathway have been identified.  The
polypeptides represent a ligand-receptor pair in a
unique costimulatory pathway which appears to be
distinct from the pathway consisting of previously
30   described proteins CD28, CTLA-4, B7.1, and B7.2.  The
polypeptides are referred to as CD28 related protein-1,
or CRP1, and B7 related protein-1, or B7RP1.

        The invention provides for nucleic acid
molecules encoding CRP1 and B7RP1 polypeptides and
35   related polypeptides.  An isolated nucleic acid

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molecule of the invention comprises a nucleotide
sequence selected from the group consisting of:

    a) the nucleotide sequence as set forth in
Figure 1A (SEQ ID NO:1);

    b) the nucleotide sequence encoding the
polypeptide from residues 1-200 or from residues 21-200
as set forth in Figure 1A (SEQ ID NO:1);

    c) a nucleotide sequence encoding a
polypeptide that is at least about 70 percent identical
to the polypeptide as set forth in Figure 1A (SEQ ID
NO: 1);

    d) a naturally occurring allelic variant or
alternate splice variant of any of (a), (b) or (c );

    e) a nucleotide sequence complementary to any
of (a), (b) or (c );

    f) a nucleotide sequence of (b),(c ) or (d)
encoding a polypeptide fragment of at least about 25,
50, 75, 100, or greater than 100 amino acid residues;

    g) a nucleotide sequence of (a), (b) or (c )
comprising a fragment of at least about 10, 15, 20, 25,
50, 75, 100, or greater than 100 nucleotides; and

    h) a nucleotide sequence which hybridizes
under stringent conditions to any of (a)-(g).


    Also provided by the invention is an isolated
nucleic acid molecule comprising a nucleotide sequence
selected from the group consisting of:

    a) the nucleotide sequence as set forth in
Figure 2A (SEQ ID NO: 6) or Figure 3A (SEQ ID NO:11) or
Figure 12A (SEQ ID NO: 16);

    b) the nucleotide sequence encoding the
polypeptide as set forth in Figure 2A (SEQ ID NO: 6)
from residues 1-322 or from residues 47-322 or as set
forth in Figure 3A (SEQ ID NO:11) from residues 1-288
or from residues 19-288, 20-288, 21-288, 22-288, 24-
288, or 28-288; or as set forth in Figure 12A from

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residues 1-302 or from residues 19-302, 20-302, 21-302, 22-302, 24-302 or 28-302;

        c) a nucleotide sequence encoding a polypeptide that is at least about 70 percent identical to the polypeptide as set forth in Figure 2A (SEQ ID NO: 6) or Figure 3A (SEQ ID NO: 11) or Figure 12A (SEQ ID NO: 16);

        d) a naturally occurring allelic variant or alternate splice variant of any of (a), (b) or (c );

        e) a nucleotide sequence complementary to any of (a), (b) or (c );

        f) a nucleotide sequence of (b),(c ) or (d) encoding a polypeptide fragment of at least about 25, 50, 75, 100, or greater than 100 amino acid residues;

        g) a nucleotide sequence of (a), (b) or (c ) comprising a fragment of at least about 10, 15, 20, 25, 50, 75, 100, or greater than 100 nucleotides; and

        h) a nucleotide sequence which hybridizes under stringent conditions to any of (a)-(g).

        The subject matter of the invention also relates to CRP1 and B7RP1 polypeptides and related polypeptides. The invention provides for an isolated polypeptide comprising the amino acid sequence selected from the group consisting of:

        a) the amino acid sequence as set forth in Figure 1A (SEQ ID NO:2);

        b) the mature amino acid sequence as set forth in Figure 1A (SEQ ID NO:2) comprising a mature amino terminus at residue 21;

        c) a fragment of the amino acid sequence set forth in Figure 1A (SEQ ID NO: 2) comprising at least about 25, 50, 75, 100, or greater than 100 amino acid residues;

        d) an ortholog of (a), (b) or (c ); and

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        e) an allelic variant or alternative splice
variant of (a), (b) or (d).


        Also in accordance with the invention is an
5   isolated polypeptide comprising the amino acid sequence
selected from the group consisting of:
        a) the amino acid sequence as set forth in
Figure 2A (SEQ ID NO: 7) or Figure 3A (SEQ ID NO:12) or
Figure 12A (SEQ ID NO: 17);
10          b) the mature amino acid sequence as set
forth in Figure 2A (SEQ ID NO: 7) comprising a mature
amino terminus at residues 47 or Figure 3A (SEQ ID
NO:12) comprising a mature amino terminus at any of
residues 19, 20, 21, 22, 24 or 28 or Figure 12A (SEQ ID
15  NO: 17) comprising a mature amino terminus at any of
residues 19, 20, 21, 22, 24, or 28;
        c) a fragment of the amino acid sequence set
forth in Figure 2A (SEQ ID NO: 7) or Figure 3A (SEQ ID
NO: 12) or Figure 12A (SEQ ID NO: 17) comprising at
20  least about 25, 50, 75, 100, or greater than 100 amino
acid residues;
        d) an ortholog of (a), (b) or (c ); and
        e) an allelic variant or alternative splice
variant of (a), (b), (c) or (d).
25

        Also encompassed by the invention are
expression vectors and host cells for production of the
polypeptides, antibodies which bind to CRP1 and B7RP1
polypeptides and to related polypeptides, and assays
30  for detecting binding of B7RP1 and B7RP1-related
polypeptides to CRP1 and CRP1-related polypeptides.
Pharmaceutical compositions comprising CRP1 or
CRP1-related polypeptides and B7RP1 or B7RP1-related
polypeptides are also encompassed by the invention.
35  Methods for identifying compounds that interact with
CRP1 or B7RP1 are also provided as are assays for

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determining whether such compounds are agonists or antagonists of CRP1 and B7RP1 activity.

CRP1 and B7RP1 polypeptides are involved in T-cell costimulation and proliferation. CRP1 and B7RP1
5   polypeptides, selective binding agents thereof, and agonists and antagonists thereof, may be useful for the diagnosis, prevention and treatment of diseases related to the control of T-cell responses.

CRP1 and B7RP1 polypeptides, selective
10   binding agents thereof, and agonists and antagonists thereof, may be useful for the diagnosis, prevention and treatment of immune disorders, either for stimulating insufficient immune response or reducing or inhibiting an exaggerated or inappropriate immune
15   response. The immune disorder may be mediated directly or indirectly by T cells.

The invention provides for treating, preventing, or ameliorating a T-cell mediated disorder comprising administering to an animal a CRP1 or B7RP1
20   polypeptide. The invention also provides for a method of diagnosing a T-cell mediated disorder or a susceptibility to a T-cell mediated disorder in an animal comprising determining the presence or amount of expression of a CRP1 or B7RP1 polypeptide; and
25   diagnosing a T-cell mediated disorder or a susceptibility to a T-cell mediated disorder based on the presence or amount of expression of the polypeptide. Typically, a T-cell mediated disorder is an immune disorder which may be mediated directly or
30   indirectly by T cells. The animal is preferably a mammal and more preferably a human The invention also provides for a method of identifying a test molecule which binds to a CRP1 or B7RP1 polypeptide comprising contacting the polypeptide with a test compound and
35   determining the extend of binding of the polypeptide to the test compound. The method may be used to identify

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agonists and antagonists of CRP1 and/or B7RP1
polypeptide.

Antagonists of CRP1 and/or B7RP1 polypeptides
may be used as immunosuppressive agents for many
5    indications, including autoimmune disorders (such as
rheumatoid arthritis, psoriasis, multiple sclerosis,
diabetes, and systemic lupus erythematosus), toxic
shock syndrome, bone marrow and organ transplantation,
inflammatory bowel disease, allosensitization due to
10   blood transfusions, and the treatment of graft vs. host
disease. In addition, antagonists may be used as
inhibitory agents for T-cell dependent B-cell mediated
indications including asthma and allergy, and antibody
mediated autoimmunity. Agonists of the CRP1 and/or
15   B7RP1 polypeptides may be useful in, but not restricted
to, T-cell activation for tumor surveillance and
removal.

An antagonist of the invention includes an
antibody, or fragment thereof, which is reactive with
20   or binds to B7RP1 or to an extracellular domain of
B7RP1 wherein the antibody reduces or eliminates the
binding to B7RP1 to CRP1. In one embodiment, the
antibody binds selectively to human B7RP1 or to an
extracellular domain thereof. The antibody or fragment
25   thereof which is an antagonist inhibits partially or
completely the immune costimulatory activity of B7RP1.
In a preferred embodiment, the antibody is a monoclonal
antibody and may be murine, human, chimeric or
humanized.

30   The invention further provides for a method
of regulating the interaction of B7RP1 with CRP1
comprising administering to an animal a selective
binding agent of CRP1 or a selective binding agent of
B7RP1 or both. In one embodiment, the selective
35   binding agent is an antibody which binds to B7RP1 and
reduces or eliminates the binding to B7RP1 to CRP1.

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The invention also provides a method of regulating immune costimulation mediated by B7RP1 comprising administering to an animal a selective binding agent of B7RP1.  The selective binding agent is preferably an

5   antibody which binds to B7RP1 and partially or completely inhibits immune costimulation mediated by B7RP1.

The invention also provides for a method of regulating T-cell activation or proliferation in an

10  animal comprising administering to the animal a nucleic acid molecule encoding a CRP1 or B7RP1 polypeptide. For example, a nucleic acid molecule encoding a B7RP1 polypeptide may be used in gene therapy to enhance T-cell activation in response to various tumors.

15  Also encompassed by the invention is a transgenic non-human mammal comprising a nucleic acid molecule encoding a CRP1 or B7RP1 polypeptide.  The CRP1 or B7RP1 nucleic acids are introduced into the mammal in a manner that allows expression and increased

20  circulation levels of CRP1 or B7RP1 polypeptides.  The transgenic non-human mammal is preferably a rodent, and more preferably a mouse or a rat.

Description of Figures

25  Figure 1.  A) DNA and amino acid sequence murine CRP1 (mCRP1).  Predicted signal sequence of CRP1 is underlined at the amino-terminus and the experimentally determined pro-peptide cleavage site is indicated by an asterisk.  Predicted transmembrane

30  sequence is underlined toward the carboxy-terminus.  B) Amino acid alignment of murine CRP1 protein sequence (mCRP1) with murine CD28 (mCD28).

Figure 2.  A) DNA and amino acid sequence of

35  murine B7RP1 (mB7RP1).  Predicted signal sequence of B7RP1 is underlined at the amino-terminus and the

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experimentally determined pro-peptide cleavage site is
indicated by an asterisk.  Predicted transmembrane
sequence is underlined toward the carboxy-terminus.  B)
Amino acid alignment of B7RP1 protein sequence (mB7RP1)
5   with murine CD80 (mCD80).


        Figure 3.  A) Structure and sequence of the
protein coding region of the putative human B7RP1
(hB7RP1).  Predicted signal sequence of hB7RP1 is
10  underlined at the amino-terminus.  Predicted signal
peptide cleavage sites are marked by asterisks.
Predicted transmembrane sequence is underlined toward
the carboxy-terminus.  B) Amino acid alignment of the
putative mature hB7RP1 protein with the mature murine
15  B7RP1 (mB7RP1) protein.


        Figure 4.  A) Expression of soluble CRP1-Fc
fusion protein from 293T cells transfected with the
pcDNA3/CRP1-Fc.  Normalized volumes of cell lysate or
20  conditioned medium were loaded and separated on a 10%
PAGE gel as indicated.  Western analysis of cell lysate
and cell media supernatant for expression of
cell-associated (cell lysate) and secreted (media) Fc
fusion proteins.  Primary antibody was Goat-anti human
25  Fc antibody (Pierce Chemical Company, Rockford, IL.).
B) Expression of soluble B7RP1-Fc fusion protein from
293T cells transfected with the pcDNA3/ B7RP1-Fc.  20
μl of normalized cell lysate or media supernatant were
loaded and separated on a 10% PAGE gel. Western
30  analysis was conducted as in (A).


        Figure 5.  Interaction of CRP1-Fc and B7RP1-
Fc fusion proteins with membrane-bound proteins
expressed in COS-7 cells.  COS-7 cells transiently
35  transfected with pcDNA3/CRP1, pcDNA3/B7RP1, or pcDNA3
vector alone.  CHO D-cells were transfected with

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psDRα/hCD28 and stably expressed human CD28 (hCD28).
Cells expressing membrane-bound CRP1, B7RP1, or hCD28,
are represented in rows as indicated at the left side
of the panel.  Fc fusion proteins were incubated with
5   the plate-bound cells in columns as indicated at the
top of the panel.  After incubation, cells were washed,
and bound Fc fusion proteins were detected using an
anti-human Fc antibody and ACAS (Adherent Cell Analysis
and Sorting; ACAS Ultima, Meridian Instruments, Inc.,
10  Okemos, MI) analysis.

Figure 6.  FACS (Fluorescence-Activated Cell
Sorter) analysis of expression of the receptor for
B7RP1 (putatively, CRP1) on activated CD4+ and CD8+
15  T-cells.  Mouse splenocytes were activated with PMA and
ionomycin for 12 hours.  B7RP1-Fc fusion protein,
control Fc protein (Mock-Fc), or PBS (no stain), were
incubated with the cells, washed, and subsequently
incubated with goat-anti-human Fc-FITC conjugated
20  antibody(GaHuFc-FITC) as indicated at the bottom of
each panel.  Cell marker antibodies (for T-cell markers
CD4 and CD8) PE conjugated, or isotype control antibody
(rat isotype) PE conjugated, or PBS (no stain), were
added as indicated at the left side of each individual
25  panel.

Figure 7.  FACS (Fluorescence-Activated Cell
Sorter) analysis of the expression of B7RP1 on B-cells.
Fluorocytometric analysis of the expression of the
30  ligand for CRP1 (presumably, B7RP1) on mouse
splenocytes.  CRP1-Fc fusion protein, control Fc
protein (Mock-Fc), or PBS (no stain), were incubated
with the cells, washed, and subsequently incubated with
goat-anti-human Fc-FITC conjugated antibody
35  (GaHuFc-FITC) as indicated at the bottom of each panel.

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PE conjugated cell marker antibody to CD45R (CD45R is a
B-cell marker) or isotype control antibody (rat
isotype), or PBS (no stain), were added as indicated at
the left side of each individual panel.

5

Figure 8.  FACS analysis of the expression of
mCRP1 ligand on peritoneal macrophages.  Peritoneal
cells were first distinguished in subsets on the ground
of their light scattering properties (panel A).
10  Macrophages were identified in region 5 (R5) because of
their ability to strongly scatter light forward (FSC)
and sideways (SSC) and because of their positive
staining for the F4/80 antigen, a marker for
macrophages (panel B).  Macrophages in region 6 (R6)
15  were singled out on the basis of their less intense
staining for the F4/80 antigen and found to be stained
by the CRP1-Fc fusion protein (presumably because of
their expression of B7RP1).

20  Figure 9.  Inhibition of T-cell proliferation
using a B7RP1-Fc fusion protein.  T-cells from mouse
splenocytes were activated by increasing concentrations
of Conconavalin A (Con A) as indicated at the bottom of
the graph.  mCRP1-Fc, mB7RP1, and mB7.2-Fc fusion
25  proteins were added to enriched T-cells from
splenocytes in the absence (no adds) or presence of Con
A.  200,000 cells were used in the T-cell proliferation
assays in a 96-well plate.  Cells were incubated with
media (no adds) or Fc fusion proteins as indicated in
30  the graph legend.  After 42 hr, cells were pulsed with
H-thymidine for 6 hr, then harvested and incorporated
radioactivity determined.  Average CPM and standard
deviation from triplicate samples are represented.

35  Figure 10.  A) Normal mesenteric lymph node
from control Mouse #10 showing the cortex, paracortex

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and medulla of the node. Hematoxylin-eosin (H&E) stain, 40x magnification. B) Markedly enlarged mesenteric lymph node from WX11 Mouse #40 with prominent follicular hyperplasia (FH), expansion of paracortex 5  and medullary cord hyperplasia (MH). H&E, 40x.  C) Close-up of the medullary cords (MC) and sinuses (MS) from the mesenteric lymph node of control Mouse #10. Note the small medullary cords composed of mostly small lymphocytes adjacent to medullary sinuses with fleshy 10  macrophages. H&E, 400x.  D. Close-up of the medullary cords (MC) and sinuses (MS) from the mesenteric lymph node of WX11 Mouse #40. Note the markedly thickened medullary cords composed of large numbers of plasma cells with occasional Russell body cells (arrow). H&E, 15  400x.  E) Normal spleen from control Mouse #10 showing red pulp and white pulp areas with periarteriolar lymphoid sheaths (PALS), 100x. *Inset*: close-up of the marginal zone surrounding the white pulp with small lymphocytes, macrophages and occasional plasma cells, 20  400x.  F) Spleen from WX11 Mouse #6 with enlarged white pulp areas, including PALS and follicles (arrow), 100x. *Inset*: close-up of the marginal zone with numerous plasma cells and occasional Russell bodies, 400x.  G) Ileum with Peyer's patch from control Mouse #25 with 25  the interfollicular zone (arrow) flanked by two secondary follicles, 40x.  H) Ileum with Peyer's patch from WX11 Mouse#32 with markedly enlarged follicles with prominent germinal centers and interfollicular tissue (arrow), 40x.

30

Figure 11.  A) Normal mesenteric lymph node from control Mouse #5 showing the cortex, paracortex and medulla of the node. Hematoxylin-eosin (H&E) stain, 40x magnification.  B) Markedly enlarged mesenteric 35  lymph node from WX11 Mouse #33 with prominent follicular hyperplasia (*top*: rows of secondary

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follicles in the outer cortex), expansion of the
paracortex (*center*) and medullary cord hyperplasia
(*bottom*). H&E, 40x.  C) Immunohistochemical staining of
the mesenteric lymph node from control Mouse #10 with
5    anti-B220 antibody (B cell marker). Note the intensely
(brown) staining cortical area and thin medullary
cords. Immunostaining performed using the avidin-biotin
complex (ABC) immunoperoxidase method (DAB chromogen,
hematoxylin counterstain), 40x.  D) Immunohistochemical
10   staining of the mesenteric lymph node from WX11 Mouse
#33 with anti-B220 antibody. Note the intensely
staining cortical follicles and medullary cords
(although the mature plasma cells in the cords are
negative for B220), 40x.  E) Immunohistochemical
15   staining of the lymph node from control Mouse #10 with
anti-CD3 antibody (T-cell marker). Note the
immunostaining of the paracortical zone of the node,
40x.  F) Immunohistochemical staining of the lymph node
from WX11 Mouse #33 with anti-CD3 antibody. Note the
20   enlarged, intensely staining paracortical areas of the
node, 40x.

        Figure 12.  A) Structure and sequence of the
protein coding region of human B7RP1 (hB7RP1).
25   Predicted signal sequence of hB7RP1 is underlined at
the amino-terminus.  Predicted signal peptide cleavage
sites are marked by asterisks. Predicted transmembrane
sequence is underlined toward the carboxy-terminus.  B)
Amino acid alignment of the putative mature hB7RP1
30   protein with the mature murine B7RP1 (mB7RP1) protein.

        Figure 13.  A) Structure and sequence of the
protein coding region of human CRP1 (hCRP1).  Predicted
signal sequence of hCRP1 is underlined at the amino-
35   terminus.  Predicted signal peptide cleavage sites are

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marked by asterisks. Predicted transmembrane sequence
is underlined toward the carboxy-terminus.   B) Amino
acid alignment of the hCRP1 protein with the murine
CRP1 (mB7RP1) protein.

5          Figure 14.   CRP-1 is on resting memory T-
cells.   Resting splenocytes from 6-7 month old mice
were double-stained using B7RP-1-Fc labeled by an FITC-
conjugated anti-human Fc antibody and a PE-conjugated
10    antibody to either CD44(Fig 14A), CD45RB(Fig 14B), or
CD69(Fig 14C).

          Figure 15.   T-cell co-stimulation by B7RP-1-
Fc fusion protein.   A) T-cell proliferation induced by
15    different quantities of B7RP-1-Fc (closed squares),
B7.2-Fc (closed circles), or OPG-Fc fusion protein
control (open squares) in conjunction with anti-CD3
antibody. Fusion proteins were used at various
concentrations to coat 96 well plates pre-coated with
20    anti-human Fc FAb$_2$ (12.5 µg/ml) and anti-CD3 antibody
(0.9 µg/ml).   B7RP-1-Fc and B7.2-Fc co-stimulate T-
cells in a dose-dependent fashion up to 0.3 µg/ml, at
which the maximal effect is achieved.   B)   T-cell
proliferation induced by B7RP-1-Fc (closed squares),
25    B7.2-Fc (closed circles), non-fused Fc (open squares),
or no Fc (open circles) in conjunction an anti-CD3
antibody (0.85 µg/ml) and in the presence of various
concentrations of a rabbit anti-B7RP-1-Fc polyclonal
antibody.   Fc fusion proteins were used at a
30    concentration of 0.3 µg/ml and were bound to the plates
as above.   The anti-B7RP-1-Fc antibody was raised to
purified B7RP-1-Fc by subcutaneous injections of
antigen emulsified in adjuvant, and then was affinity
purified. The antibodies were incubated for 30 min with
35    the Fc fusion proteins before the addition of the
cells.   The anti-B7RP-1-Fc antibody specifically

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inhibits the T-cell proliferation induced by B7RP-1-Fc
in a dose-dependent fashion.

Figure 16.  Effect of CRP-1-Fc and B7RP-1-Fc
5    proteins on the incidence (A) and severity (B) of
collagen induced arthritis in mice. Collagen induced
arthritis susceptible B10.RIII mice were immunized at
the base of the tail with 10 µg porcine collagen type
II in CFA. Mice received 100 µg of fusion protein twice
10   per week.  Fc fusion proteins and control PBS treatment
are indicated in the figure legend.

Figure 17. Proximal Colon in B7RP-1-Fc
Transgenic Mice.  (A) Normal proximal colon from
15   control Mouse#53F (female) showing the gut wall with
mucosa, submucosa, muscularis and serosa. Hematoxylin-
eosin (H&E) stain, 40x magnification.  (B) Diffusely
thickened proximal colon from B7RP-1-Fc transgenic
Mouse#111F with prominent glandular hypertrophy,
20   fissuring ulceration and transmural inflammation. H&E,
40x.  (C) Lower power view of proximal colon (as in
panel B) from B7RP-1-Fc transgenic Mouse#111F with
multifocal fissuring ulceration and transmural
inflammation. H&E, 20x.  (D) Close-up of the fissuring
25   ulcer and hypertrophic colonic glands from B7RP-1-Fc
transgenic Mouse#111F (shown in panels B and C above).
Note the lumen with mucopurulent exudate. H&E, 100x.
(E) Close-up of granulomatous inflammation in the
submucosa of B7RP-1-Fc transgenic Mouse#112F with a
30   multinucleated giant cell surrounded by macrophages,
lymphocytes and fewer neutrophils. H&E, 400x.  (F)
Close-up of granulomatous inflammation in the mucosa of
B7RP-1-Fc transgenic mouse #112F with epithelioid
macrophages mixed with lymphocytes, plasma cells and

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fewer neutrophils subjacent to mucosal glands. H&E,
400x.

Figure 18. Distal Colon in B7RP-1-Fc
5    Transgenic Mice.   (A) Normal distal colon from control
Mouse#53F (female) showing the layers of the gut wall
with mucosa, submucosa, muscularis and serosa.
Hematoxylin-eosin (H&E) stain, 40x magnification.   (B)
Diffusely thickened distal colon from B7RP-1-Fc
10   transgenic Mouse#111F (female) with prominent glandular
hypertrophy and hyperplasia and scattered crypt
abscesses. H&E, 40x.   (C) Diffusely thickened distal
colon from B7RP-1-Fc transgenic Mouse#55M (male) with
prominent glandular hypertrophy and hyperplasia. H&E,
15   40x.   (D) Diffusely thickened distal colon from B7RP-1-
Fc transgenic Mouse#112F (female) with hypertrophic
colonic glands, focal lymphoid aggregates and many
crypt abscesses. H&E, 40x.   (E) Immunohistochemical
staining of the distal colon from B7RP-1-Fc transgenic
20   Mouse#112F with anti-CD3 antibody (T cell marker). Note
the immunostaining of the superficial mucosa and
colonic lymphoid patch. H&E, 40x.   (F) Close-up of the
colonic mucosa of B7RP-1-Fc transgenic Mouse#112F with
a crypt abscess (arrow) and lymphoid aggregate composed
25   of B220+ B cells (inset). H&E, 100x.

Figure 19. Small Intestine in B7RP-1-Fc
Transgenic Mice.   (A) Normal duodenum from control
Mouse#53F (female) showing the lumen, villi and crypts
30   of the mucosa and underlying submucosa, muscularis and
serosa. Hematoxylin-eosin (H&E) stain, 40x
magnification.   (B) Diffusely thickened duodenum from
B7RP-1-Fc transgenic Mouse#51F (female) with prominent
crypt hypertrophy and hyperplasia and mild
35   lymphoplasmacytic infiltrate in the lamina propria.
H&E, 40x.   (C) Normal jejunum from control mouse #53F

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(female) showing the normal length of the villi and
crypts in the jejunal mucosa. H&E, 40x magnification.
(D) Markedly thickened jejunal mucosa from B7RP-1-Fc
transgenic Mouse#51F (female) with locally extensive
5    crypt hypertrophy and hyperplasia. H&E, 40x.    (E)
Normal ileum from control Mouse#53F (female) showing
the normal length of the villi and crypts in the ileal
mucosa. H&E, 40x magnification.    (F) Mild atrophy of
ileal mucosa from B7RP-1-Fc transgenic Mouse#231M
10    (male) with focal loss and blunting of villi. H&E, 40x.

        Figure 20.    The B7RP-1-Fc fusion protein
inhibits tumor growth in mice. Meth A sarcoma cells
were implanted intradermally in the abdomen of Balb/C
15    mice.    On days 7, 10, 14, and 17, after implantation,
the mice were treated with vehicle (dark diamonds) or
murine B7RP-1-Fc (grey triangles, Example 7). Tumor
volume was measured, as described in Example 20, on the
indicated days after implantation. The tumor growth was
20    monitored up to day 28.    Each group had eight mice.

        Figure 21.    T-cell co-stimulation by human
B7RP-1-Fc.    Anti-CD3 and human B7RP-1 Fc were used to
coat 96 well plates, and $1 \times 10^5$ T-cells/well (>98%
25    CD3+) were cultured and harvested as described in
Example 21.    A) Co-stimulation induced by anti-CD3 only
(closed circles), 0.5 µg/ml B7RP-1 Fc (closed
triangles), 0.5 µg/ml OPG-Fc (open circles), and 5
µg/ml anti-CD28 (open triangles) at different
30    concentrations of anti-CD3 primary stimulation. Data
show that B7RP-1-Fc co-stimulated anti-CD3 primed T-
cells to similar levels as co-stimulation using anti-
CD28 antibodies. Data shown are mean [$^3$H]TdR
incorporated +/- SD in triplicate wells from one
35    representative experiment of several experiments

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generated with T-cells isolated from three normal
donors.  B) Dose-dependent inhibition of B7RP-1-Fc co-
stimulation by CRP-1-Fc.  T-cells were cultured in
wells coated with both anti-CD3 at 0.3 μg/ml and 0.5

5      μg/ml B7RP-1-Fc.  Serially diluted concentrations of
CRP-1-Fc (closed circles) or OPG-Fc (open circles) were
preincubated with the B7RP-1-Fc for 30 min prior to the
addition of T-cells.  Data show that CRP-1-Fc inhibits
B7RP-1 induced co-stimulation in a dose-dependent

10     manner. Percent inhibition is plotted against CRP-1-Fc
or OPG-Fc protein concentration. Data shown are mean
[$^3$H]TdR incorporated +/- SD of three experiments done
in triplicate wells and are representative of
experiments generated with two normal donors. C) Co-

15     stimulation by CHO human B7RP-1 cells.  T-cells were
purified from peripheral blood and were cultured with
various concentrations of anti-CD3 in the presence of
anti-CD3 alone (closed circles), 1 x 10$^4$ CHO vector
control cells (open circles) or 1 x 10$^4$ CHO B7RP-1

20     cells (closed triangles), as described in Example 22.
The data show that membrane-bound B7RP-1 co-stimulated
T-cell growth to a level similar to that observed using
B7RP-1-Fc fusion proteins.  Data shown are the mean +/-
SD of triplicate cultures and are representative of

25     results generated with two normal donors.
D) Cytokine production.  T-cells were cultured as
described in (Figure 21A) and supernatants were
collected at 48 (black bars) and 72 (gray bar) hrs.
Data show that the amount of IL-2 produced by B7RP-1-Fc

30     co-stimulated cells (top graph) was similar to that
produced by cells stimulated by anti-CD3 and control Fc,
but significantly less than that produced by anti-CD28
co-stimulated cells.  Data also show that B7RP-1-Fc co-
stimulation enhanced IL-10 (middle graph) and IFN-gamma

35     (bottom graph) production.

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Detailed Description of the Invention

The invention provides for novel polypeptides
referred to herein as CRP1 and B7RP1, which comprise a
receptor-ligand pair that is involved in T-cell
activation. cDNAs encoding the polypeptides were
identified from a library prepared from mouse
intestinal intraepithelial cells and screened on the
basis of homology to the CD28 and CTLA-4 polypeptides
(for CRP1) or B7.1 and B7.2 polypeptides (for B7RP1).

CD28 related protein-1, or CRP1, is predicted
to be a type I transmembrane protein with a signal
sequence and extracellular domain at the amino-
terminus, a transmembrane domain, and a carboxy
terminal intracellular domain (Figure 1). The full-
length CRP1 protein is 180 amino acids in its mature
form. The predicted leader sequence spans about amino
acid residues 1-20 (relative to the initiating
methionine) and the extracellular domain of the mature
protein encompasses about residues 21-145 (Example 1).
The predicted transmembrane domain spans about residues
146-163 and the intracellular domain encompasses about
residues 164-200. The amino terminal extracellular
domain is similar to an Ig loop with conserved putative
intra- and inter-molecular bonding cysteines.
Furthermore, a "MYPPPY" motif, which is previously
known to be important for B7.1 and B7.2 binding to CD28
and CTLA-4, is also partially conserved.

CD28 and CTLA-4 are weakly homologous as
exemplified by the 26% amino acid identity between
murine CD28 and CTLA-4. There is 19% amino acid
identity of CRP1 with murine CD28 and 14% identity of
CRP1 with murine CTLA-4. However, critical cysteine
residues are conserved between murine CD28, CTLA-4 and
CRP1 at residues 42, 63, 83, 109, and 137 (relative to

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the initiating methionine in the CRP1 protein, See
Figure 1A).  The approximate mature protein lengths and
locations of the transmembrane region relative to the
carboxy terminus are also similar in CRP1, CD28, and
5   CTLA-4.

Human CRP1 is a transmembrane protein having
the nucleotide and amino acid sequence as shown in
Figure 13A.  The predicted leader sequence spans about
residues 1-19 or about residues 1-20.  The predicted
10  mature amino terminus is at residues 20 or 21.
Preferably, the mature amino terminus is at position
21.  The extracellular domain spans from any of the
prediced mature amino termini to about amino acid
residue 140, the transmembrane domain spans about
15  residues 141-161 and the intracellular domain spans
about residues 162-199.  Human CRP1 protein has 69%
identity to the murine protein and the corresponding
nucleotide sequences are 77% identical.  The sequence
of human CRP1 was reported in Hutloff et al. Nature
20  _397_, 263-266 (1999).

B7 related protein-1, or B7RP1, is predicted
to be a type I transmembrane protein with a signal
sequence and extracellular domain at the
amino-terminus, a transmembrane domain, and a carboxy
25  terminal intracellular domain (Figure 2A).  The
full-length B7RP1 protein is 276 amino acids in its
mature form.  The predicted leader sequence spans about
amino acid residues 1-46 (relative to the initiating
methionine) and the extracellular domain of the mature
30  protein encompasses residues 47-279 (Example 3).  The
predicted transmembrane domain spans residues 280-298
and the intracellular domain encompasses residues
299-322.  Similar to B7.1 and B7.2, the extracellular
domain of B7RP1 comprises two Ig loops.

35      B7.1 and B7.2 are weakly homologous as
exemplified by the 24% amino acid identity between

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murine B7.1 and B7.2.  There is 20% amino acid identity
of B7RP1 with murine B7.1 and 19% identity of B7RP1
with murine B7.2.  However, critical cysteine residues
are conserved between murine B7.1, B7.2 and B7RP1 at
5    residues 62, 138, 185, and 242 (relative to the
initiating methionine in the B7RP1 protein, Figure 2A).
The approximate mature protein length and location of
the transmembrane region relative to the carboxy
terminus are also similar in mB7RP1, B7.1, and B7.2.
10          Human B7RP1 is also a transmembrane protein
with conserved cysteine residue in the extracellular
domain which are necessary for Ig loop structures.  The
predicted leader sequence encompasses about residues 1-
18, 1-19, 1-20, 1-21, 1-23 or 1-27 as shown in Figure
15   3A.  The predicted mature amino terminus may be at any
of the residues 19, 20, 21, 22, 24 or 28.  Preferably,
the amino terminus is at position 19.  The
extracellular domain spans from any of the mature amino
termini to about amino acid residue 259.  The predicted
20   transmembrane domain spans about residues 259-274.  The
intracellular domain encompasses residues 275-302.  The
full-length human B7RP1 nucleotide and amino acid
sequence is shown in Figure 12A.  Human B7RP1 is about
43% identical to the murine protein.
25          CRP1 and B7RP1 bind each other, but CRP1 does
not detectably bind to the B7RP1 related protein B7.2;
and B7RP1 does not exhibit detectable binding to
CRP1-related CD28 or CTLA-4 (Example 8).  B7RP1 was
shown to regulate T-cell proliferation, presumably
30   through the interaction of B7RP1 with CRP1 receptors
(Example 11).  Thus, CRP1 and B7RP1 represent a novel
pathway for regulating T-cell proliferation and
activation.
       The interaction of B7RP1 with CRP1 can be
35   regulated in such a manner that immune costimulation
and T-cell proliferation and activation can be

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increased or decreased. By way of example, anti-B7RP1
monoclonal and polyclonal antibodies raised against
murine B7RP1 blocked the B7RP1/CRP1 interaction and
also blocked T-cell proliferation induced by a B7RP1-Fc
5   fusion protein (see Example 17). A human CRP-1-Fc
fusion protein blocked human T-cell proliferation
induced by human B7RP-1-Fc (Example 21). In addition,
addition of a CRP1-Fc fusion protein delayed the onset
of arthritic symptoms in a mouse model of rheumatoid
10  arthritis (see Example 18). B7RP-1/CRP-1 co-
stimulation can also be increased by addition of B7RP-
1-Fc fusion protein or other activators of this pathway
(Example 20).


15

Nucleic Acid Molecules
        The term "isolated nucleic acid molecule"
refers to a nucleic acid molecule that is free from at
least one contaminating nucleic acid molecule with
20  which it is naturally associated, and preferably
substantially free from any other contaminating
mammalian nucleic acid molecules.
        The term "allelic variant" refers to one of
several possible naturally occurring alternate forms of
25  a gene occupying a given locus on a chromosome of an
organism.
        The term "splice variant" refers to a nucleic
acid molecule, usually RNA, which is generated by
alternative processing of intron sequences in an RNA
30  transcript.
        The term "high stringency conditions" refers
to those conditions which: (1) employ low ionic
strength and high temperature for washing, for example,
0.1 X SSC (0.015 M NaCl/0.0015 M sodium citrate) 0.1%
35  NaDodSO$_4$ (SDS) at 50°C, or (2) employ during

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hybridization a denaturing agent such as formamide, for example, 50% (vol/vol) formamide with 0.1% bovine serum albumin/0.1%. Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 5 X SSC (750 mM

5   NaCl, 75 mM sodium citrate) at 42°C. Another example of high stringency conditions is 50% formamide, 5 x SSC, 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran

10  sulfate at 42°C, with washes at 42°C in 0.2 x SSC and 0.1% SDS.

    The term "moderate stringency conditions" refers to those conditions which include the use of a washing solution and hybridization conditions (e.g.,

15  temperature and ionic strength) less stringent than described above. An example of moderately stringent conditions are conditions such as overnight incubation at 37°C in a solution comprising: 20% formamide, 5 x SSC, 50 mM sodium phosphate (pH 7.6), 5 x Denhardt's

20  solution, 10% dextran sulfate, and 20 µl/ml denatured sheared salmon sperm DNA, followed by washing the filters in 1 x SSC at about 37-50°C. The skilled artisan will recognize how to adjust the temperature, ionic strength and other parameters as necessary to

25  accommodate factors such as probe length and the like.

    Recombinant DNA technology methods are set forth in Sambrook *et al.* (*Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989)) and/or Ausubel *et al.*,

30  eds., (*Current Protocols in Molecular Biology*, Green Publishers Inc. and Wiley and Sons, NY (1994)) which are hereby incorporated by reference in their entirety.

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The invention provides for isolated nucleic acid molecules encoding CRP1 and B7RP1 polypeptides. Also provided for are nucleic acid molecules which are fragments, allelic variants, splice variants, or are

5   complementary in sequence to molecules encoding CRP1 and B7RP1 polypeptides. Nucleic acid molecules which are at least about 70% identical to molecules encoding CRP1 or B7RP1 or which hybridize to molecules encoding CRP1 or B7RP1 under moderate or high stringency

10  conditions are also encompassed. The nucleic acid molecules may be cDNA, genomic DNA, RNA or a partially or totally synthetic nucleic acid molecule. In preferred embodiments, nucleic acid molecules of the invention are at least about 75%, 80%, 85%, 90% or 95%

15  identical to nucleic acid molecules encoding CRP1 or B7RP1.

A gene or cDNA encoding a CRP1 or B7RP1 polypeptide or fragment thereof may be obtained, for example, by hybridization screening or PCR

20  amplification of a genomic or cDNA library. Probes or primers useful for screening the library can be generated based on sequence information for other known genes or gene fragments from the same or a related family of genes, for example, conserved motifs found in

25  CRP1 or B7RP1 related polypeptides such as a conserved array of cysteine residues. In addition, where a gene encoding a CRP1 or B7RP1 polypeptide has been identified from one species, all or a portion of that gene may be used as a probe to identify homologous

30  genes from other species. The probes or primers may be used to screen cDNA libraries from various tissue sources believed to express the CRP1 or B7RP1 gene.

Where oligonucleotide probes are used to screen cDNA or genomic libraries, one of the following

35  two high stringency solutions may be used. The first of these is 6 X SSC with 0.05% sodium pyrophosphate at

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35°C-62°C, with the temperature depending on the length
of the oligonucleotide probe. For example, 14 base
pair probes are washed at 35-40°C, 17 base pair probes
are washed at 45-50°C, 20 base pair probes are washed
5    at 52-57°C, and 23 base pair probes are washed at 57-
63°C. The temperature can be increased 2-3°C where the
background non-specific binding appears high. A second
high stringency solution utilizes tetramethylammonium
chloride (TMAC) for washing oligonucleotide probes.
10   One stringent washing solution is  3 M TMAC, 50 mM
Tris-HCl, pH 8.0, and 0.2% SDS. The washing
temperature using this solution is a function of the
length of the probe. For example, a 17 base pair probe
is washed at about 45-50°C.
15   Another means to prepare a gene encoding a CRP1 or
B7RP1 polypeptide or fragment thereof is to employ
chemical synthesis using methods well known to the
skilled artisan such as those described by Engels *et
al.*(*Agnew. Chem. Intl. Ed.*, 28:716-734 (1989)). These
20   methods include, *inter alia*, the phosphotriester,
phosphoramidite, and H-phosphonate methods for nucleic
acid synthesis. A preferred method for such chemical
synthesis is polymer-supported synthesis using standard
phosphoramidite chemistry. Typically, DNA encoding a
25   CRP1 or B7RP1 polypeptide will be several hundred
nucleotides in length. Nucleic acids larger than about
100 nucleotides can be synthesized as several fragments
using these methods. The fragments can then be ligated
together to form a full length CRP1 or B7RP1
30   polypeptide. Usually, the DNA fragment encoding the
amino terminus of the polypeptide will have an ATG,
which encodes a methionine residue. This methionine
may or may not be present on the mature form of a CRP1
or B7RP1 polypeptide, depending on whether the
35   polypeptide produced in the host cell is designed  to
be secreted from that cell.

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CRP1 or B7RP1 nucleic acid molecules,
fragments, and/or derivatives that do not themselves
encode polypeptides that are biologically active may
nonetheless be useful as hybridization probes in
5   diagnostic assays to test, either qualitatively or
quantitatively, for the presence of CRP1 or B7RP1 DNA
or corresponding RNA in mammalian tissue or bodily
fluid samples.

In some cases, it may be desirable to prepare
10   nucleic acid and/or amino acid variants of naturally
occurring CRP1 or B7RP1 polypeptides. Nucleic acid
variants may be produced using site directed
mutagenesis, PCR amplification, or other appropriate
methods, where the primer(s) have the desired point
15   mutations (see Sambrook *et al.*, *supra*, and Ausubel *et
al.*, *supra*, for descriptions of mutagenesis
techniques). Chemical synthesis using methods
described by Engels *et al.*, *supra*, may also be used to
prepare such variants. Other methods known to the
20   skilled artisan may be used as well.

Preferred nucleic acid variants include those
containing codons which have been altered for optimal
expression of CRP1 and B7RP1 polypeptides in a given
host cell. Particular codon alterations will depend
25   upon the selection of protein and host cell. Such
"codon optimization" can in one instance, be carried
out by selecting codons which are preferentially used
in highly expressed genes in a given host cell.
Computer algorithms which incorporate codon frequency
30   tables such as "Ecohigh. Cod" for codon preference of
highly expressed bacterial genes may be used and are
provided by the University of Wisconsin Package Version
9.0, Genetics Computer Group, Madison, WI. Other
useful codon frequency tables include
35   "Celegans_high.cod", "Celegans_low.cod",
"Drosophila_high.cod", "Human_high.cod",

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"Maize_high.cod", and "Yeast_high.cod". Other
preferred variants are those encoding conservative
amino acid changes as described below (e.g., wherein
the charge or polarity of the naturally occurring amino
5    acid side chain is not altered substantially by
substitution with a different amino acid) as compared
to wild type, and/or those designed to either generate
a novel glycosylation and/or phosphorylation site(s),
or those designed to delete an existing glycosylation
10   and/or phosphorylation site(s).

The gene, cDNA, or fragment thereof encoding
a CRP1 or B7RP1 polypeptide can be inserted into an
appropriate expression or amplification vector using
standard ligation techniques. The vector is typically
15   selected to be functional in the particular host cell
employed (i.e., the vector is compatible with the host
cell machinery such that amplification of the gene
and/or expression of the gene can occur). The gene,
cDNA or fragment thereof encoding the CRP1 or B7RP1
20   polypeptide may be amplified/expressed in prokaryotic,
yeast, insect (baculovirus systems) and/or eukaryotic
host cells. Selection of the host cell will depend in
part on whether the CRP1 or B7RP1 polypeptide or
fragment thereof is to be glycosylated and/or
25   phosphorylated. If so, yeast, insect, or mammalian
host cells are preferable.

Typically, expression vectors used in any of
the host cells will contain sequences for plasmid
maintenance and cloning and expression of inserted
30   nucleotide sequences. Such sequences, referred to
collectively as "flanking sequences", will include a
promoter and other regulatory elements such as an
enhancer(s), an origin of replication element, a
transcriptional termination element, a complete intron
35   sequence containing a donor and acceptor splice site, a
signal peptide sequence, a ribosome binding site

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element, a polyadenylation sequence, a polylinker
region for inserting the nucleic acid encoding the
polypeptide to be expressed, and a selectable marker
element. Each of these elements is discussed below.

5      Optionally, the vector may contain a "tag" sequence,
i.e., an oligonucleotide molecule located at the 5' or
3' end of a CRP1 or B7RP1 polypeptide coding sequence;
the oligonucleotide molecule encodes polyHis (such as
hexaHis), or other "tag" such as FLAG, HA (hemaglutinin

10     Influenza virus) or myc for which commercially
available antibodies exist. This tag is typically
fused to the polypeptide upon expression of the
polypeptide, and can serve as means for affinity
purification of a CRP1 or B7RP1 polypeptide from the

15     host cell. Affinity purification can be accomplished,
for example, by column chromatography using antibodies
against the tag as an affinity matrix. Optionally, the
tag can subsequently be removed from a purified CRP1 or
B7RP1 polypeptide by various means such as using

20     certain peptidases.

The human immunoglobulin hinge and Fc region
may be fused at either the N-terminus or C-terminus of
a CRP1 or B7RP1 polypeptide by one skilled in the art.
The subsequent Fc-fusion protein can be purified by use

25     of a Protein A affinity column. An immunoglobin Fc
region is known to exhibit a long pharmacokinetic
half-life in vivo and proteins fused to an Fc region
have been found to exhibit a substantially greater
half-life in vivo compared to the unfused counterpart.

30     Also, fusion to the Fc region allows for dimerization
and/or multimerization of the molecule that may be
useful for the bioactivity of some molecules.

The flanking sequence may be homologous
(i.e., from the same species and/or strain as the host

35     cell), heterologous (i.e., from a species other than
the host cell species or strain), hybrid (i.e., a

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combination of flanking sequences from more than one
source), synthetic, or it may be the native CRP1 or
B7RP1 nucleic acid flanking sequences. As such, the
source of the flanking sequence may be any unicellular
5    prokaryotic or eukaryotic organism, any vertebrate or
invertebrate organism, or any plant, provided that the
flanking sequence is functional in, and can be
activated by, the host cell machinery.

The flanking sequences useful in the vectors
10   of this invention may be obtained by any of several
methods well known in the art. Typically, flanking
sequences useful herein other than CRP1 or B7RP1
nucleic acid flanking sequence will have been
previously identified by mapping and/or by restriction
15   endonuclease digestion and can thus be isolated from
the proper tissue source using the appropriate
restriction endonucleases. In some cases, the full
nucleotide sequence of the flanking sequence may be
known. Here, the flanking sequence may be synthesized
20   using the methods described above for nucleic acid
synthesis or cloning.

Where all or only a portion of the flanking
sequence is known, it may be obtained using PCR and/or
by screening a genomic library with suitable
25   oligonucleotide and/or flanking sequence fragments from
the same or another species.

Where the flanking sequence is not known, a
fragment of DNA containing a flanking sequence may be
isolated from a larger piece of DNA that may contain,
30   for example, a coding sequence or even another gene or
genes. Isolation may be accomplished by restriction
endonuclease digestion using one or more carefully
selected enzymes to isolate the proper DNA fragment.
After digestion, the desired fragment may be isolated
35   by agarose gel purification, Qiagen® column or other
methods known to the skilled artisan. Selection of

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suitable enzymes to accomplish this purpose will be
readily apparent to one of ordinary skill in the art.

    The origin of replication element is
typically a part of prokaryotic expression vectors
5  purchased commercially, and aids in the amplification
of the vector in a host cell. Amplification of the
vector to a certain copy number can, in some cases, be
important for optimal expression of the CRP1 or B7RP1
polypeptide. If the vector of choice does not contain
10  an origin of replication site, one may be chemically
synthesized based on a known sequence, and ligated into
the vector.

    The transcription termination element is
typically located 3' of the end of a CRP1 or B7RP1
15  polypeptide coding sequence and serves to terminate
transcription of a CRP1 or B7RP1 polypeptide. Usually,
the transcription termination element in prokaryotic
cells is a G-C rich fragment followed by a poly T
sequence. While the element is easily cloned from a
20  library or even purchased commercially as part of a
vector, it can also be readily synthesized using
methods for nucleic acid synthesis such as those
described above.

    A selectable marker gene element encodes a
25  protein necessary for the survival and growth of a host
cell grown in a selective culture medium. Typical
selection marker genes encode proteins that (a) confer
resistance to antibiotics or other toxins, e.g.,
ampicillin, tetracycline, or kanamycin for prokaryotic
30  host cells, (b) complement auxotrophic deficiencies of
the cell; or (c) supply critical nutrients not
available from complex media. Preferred selectable
markers are the kanamycin resistance gene, the
ampicillin resistance gene, and the tetracycline
35  resistance gene.

The ribosome binding element, commonly called the Shine-Dalgarno sequence (prokaryotes) or the Kozak sequence (eukaryotes), is usually necessary for translational initiation of mRNA. The element is typically located 3' to the promoter and 5' to the coding sequence of the CRP1 or B7RP1 polypeptide to be synthesized. The Shine-Dalgarno sequence is varied but is typically a polypurine (i.e., having a high A-G content). Many Shine-Dalgarno sequences have been identified, each of which can be readily synthesized using methods set forth above and used in a prokaryotic vector.

In those cases where it is desirable for a CRP1 or B7RP1 polypeptide to be secreted from the host cell, a signal sequence may be used to direct export of the polypeptide from the host cell. A CRP1 or B7RP1 transmembrane domain is also inactivated by mutation or deletion to prevent attachment to the host membrane. Typically, the signal sequence is positioned in the coding region of a CRP1 or B7RP1 gene or cDNA, or directly at the 5' end of a CRP1 or B7RP1 gene coding region. Many signal sequences have been identified, and any of them that are functional in the selected host cell may be used in conjunction with a CRP1 or B7RP1 gene or cDNA. Therefore, the signal sequence may be homologous or heterologous to a CRP1 or B7RP1 gene or cDNA, and may be homologous or heterologous to a CRP1 or B7RP1 polypeptides gene or cDNA. Additionally, the signal sequence may be chemically synthesized using methods set forth above.

In most cases, secretion of the polypeptide from the host cell via the presence of a signal peptide will result in the removal of the amino terminal methionine from the polypeptide.

In many cases, transcription of a CRP1 or B7RP1 gene or cDNA is increased by the presence of one

or more introns in the vector; this is particularly
true where a CRP1 or B7RP1 polypeptide is produced in
eukaryotic host cells, especially mammalian host cells.
The introns used may be naturally occurring within a
5    CRP1 or B7RP1 gene, especially where the gene used is a
full length genomic sequence or a fragment thereof.
Where the intron is not naturally occurring within the
gene (as for most cDNAs), the intron(s) may be obtained
from another source.  The position of the intron with
10   respect to the 5' flanking sequence and a CRP1 or B7RP1
gene is generally important, as the intron must be
transcribed to be effective.  As such, where a CRP1 or
B7RP1 gene inserted into the expression vector is a
cDNA molecule, the preferred position for the intron is
15   3' to the transcription start site, and 5' to the polyA
transcription termination sequence.  Preferably the
intron or introns will be located on one side or the
other (i.e., 5' or 3') of the cDNA such that it does
not interrupt the this coding sequence.  Any intron
20   from any source, including any viral, prokaryotic and
eukaryotic (plant or animal) organisms, may be used to
practice this invention, provided that it is compatible
with the host cell(s) into which it is inserted.  Also
included herein are synthetic introns.  Optionally,
25   more than one intron may be used in the vector.
           Where one or more of the elements set forth
above are not already present in the vector to be used,
they may be individually obtained and ligated into the
vector.  Methods used for obtaining each of the
30   elements are well known to the skilled artisan.
           Preferred vectors for practicing this
invention are those which are compatible with
bacterial, insect, and mammalian host cells.  Such
vectors include, inter alia, pCRII, pCR3, and pcDNA3.1
35   (Invitrogen Company, San Diego, CA), pBSII (Stratagene
Company, La Jolla, CA), pET15b (Novagen, Madison, WI),

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pGEX (Pharmacia Biotech, Piscataway, NJ), pEGFP-N2
(Clontech, Palo Alto, CA), pETL (BlueBacII;
Invitrogen), and pFastBacDual (Gibco/BRL, Grand Island,
NY).

5          After the vector has been constructed and a
nucleic acid molecule encoding a full length or
truncated CRP1 or B7RP1 polypeptide has been inserted
into the proper site of the vector, the completed
vector may be inserted into a suitable host cell for
10   amplification and/or polypeptide expression.
           Host cells may be prokaryotic host cells
(such as E. coli) or eukaryotic host cells (such as a
yeast cell, an insect cell, or a vertebrate cell). The
host cell, when cultured under appropriate conditions,
15   can synthesize a CRP1 or B7RP1 polypeptide which can
subsequently be collected from the culture medium (if
the host cell secretes it into the medium) or directly
from the host cell producing it (if it is not
secreted). After collection, a CRP1 or B7RP1
20   polypeptide can be purified using methods such as
molecular sieve chromatography, affinity
chromatography, and the like.
           Selection of the appropriate host cell for
CRP1 or B7RP1 polypeptide production will depend on
25   various factors, such as desired expression levels,
polypeptide modifications that are required for
activity, such as glycosylation or phosphorylation, or
ease of folding into a biologically active molecule.
           Suitable cells or cell lines may be mammalian
30   cells, such as Chinese hamster ovary cells (CHO), human
embryonic kidney (HEK) 293 or 293T cells, or 3T3 cells.
The selection of suitable mammalian host cells and
methods for transformation, culture, amplification,
screening and product production and purification are
35   known in the art. Other suitable mammalian cell lines,
are the monkey COS-1 and COS-7 cell lines, and the CV-1

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cell line. Further exemplary mammalian host cells include primate cell lines and rodent cell lines, including transformed cell lines. Normal diploid cells, cell strains derived from *in vitro* culture of
5 primary tissue, as well as primary explants, are also suitable. Candidate cells may be genotypically deficient in the selection gene, or may contain a dominantly acting selection gene. Other suitable mammalian cell lines include but are not limited to,
10 mouse neuroblastoma N2A cells, HeLa, mouse L-929 cells, 3T3 lines derived from Swiss, Balb-c or NIH mice, BHK or HaK hamster cell lines.

Similarly useful as host cells are bacterial cells. For example, the various strains of *E. coli*
15 (e.g., HB101, DH5α, DH10, and MC1061) are well-known as host cells in the field of biotechnology. Various strains of *B. subtilis*, *Pseudomonas spp.*, other *Bacillus spp.*, *Streptomyces spp.*, and the like may also be employed in this method.

20 Many strains of yeast cells known to those skilled in the art are also available as host cells for expression of the polypeptides of the present invention.

Additionally, where desired, insect cell
25 systems may be utilized in the methods of the present invention. Such systems are described for example in Kitts *et al.* (*Biotechniques*, 14:810-817 (1993)), Lucklow (*Curr. Opin. Biotechnol.*, 4:564-572 (1993)) and Lucklow *et al.* (*J. Virol.*, 67:4566-4579 (1993)).
30 Preferred insect cells are Sf-9 and Hi5 (Invitrogen, Carlsbad, CA).

"Transformation" or "transfection" of an expression vector into the selected host cell may be accomplished using such methods as calcium chloride,
35 electroporation, microinjection, lipofection or the

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DEAE-dextran method.  The method selected will in part be a function of the type of host cell to be used. These methods and other suitable methods are well known to the skilled artisan, and are set forth, for example,
5   in Sambrook *et al.*, *supra*.

The host cells transformed or transfected with an expression vector may be cultured using standard media well known to the skilled artisan.  The media will usually contain all nutrients necessary for
10   the growth and survival of the cells.  Suitable media for culturing *E. coli* cells are, for example, Luria Broth (LB) and/or Terrific Broth (TB).  Suitable media for culturing eukaryotic cells are RPMI 1640, MEM, DMEM, all of which may be supplemented with serum
15   and/or growth factors as required by the particular cell line being cultured.  A suitable medium for insect cultures is Grace's medium supplemented with yeastolate, lactalbumin hydrolysate, and/or fetal calf serum as necessary.

20   Typically, an antibiotic or other compound useful for selective growth of the transformed cells only is added as a supplement to the media.  The compound to be used will be dictated by the selectable marker element present on the plasmid with which the
25   host cell was transformed.  For example, where the selectable marker element is kanamycin resistance, the compound added to the culture medium will be kanamycin.

The amount of CRP1 or B7RP1 polypeptide produced in the host cell can be evaluated using
30   standard methods known in the art.  Such methods include, without limitation, Western blot analysis, SDS-polyacrylamide gel electrophoresis, non-denaturing gel electrophoresis, HPLC separation, immunoprecipitation, and/or activity assays such as DNA
35   binding gel shift assays.

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If a CRP1 or B7RP1 polypeptide has been
designed to be secreted from the host cells, the
majority of polypeptide may be found in the cell
culture medium.  Polypeptides prepared in this way will
5   typically not possess an amino terminal methionine, as
it is removed during secretion from the cell.  If,
however, a CRP1 or B7RP1 polypeptide is not secreted
from the host cells, it will be present in the
cytoplasm and/or the nucleus (for eukaryotic host
10  cells) or in the cytosol (for gram negative bacteria
host cells) and may have an amino terminal methionine.
Purification of a CRP1 or B7RP1 polypeptide
from solution can be accomplished using a variety of
techniques.  If the polypeptide has been synthesized
15  such that it contains a tag such as Hexahistidine (CRP1
or B7RP1/hexaHis) or other small peptide such as FLAG
(Eastman Kodak Co., New Haven, CT) or myc (Invitrogen,
Carlsbad, CA) at either its carboxyl or amino terminus,
it may essentially be purified in a one-step process by
20  passing the tagged polypeptide through an affinity
column where the column matrix has a high affinity for
the tag or for the polypeptide directly (i.e., a
monoclonal antibody specifically recognizing a CRP1 or
B7RP1 polypeptide).  For example, polyhistidine binds
25  with great affinity and specificity to nickel, thus an
affinity column of nickel (such as the Qiagen® nickel
columns) can be used for purification of CRP1 or
B7RP1/polyHis.  (See for example, Ausubel et al., eds.,
Current Protocols in Molecular Biology, Section
30  10.11.8, John Wiley & Sons, New York (1993)).
Where a CRP1 or B7RP1 polypeptide is prepared
without a tag attached, and no antibodies are
available, other well known procedures for purification
can be used.  Such procedures include, without
35  limitation, ion exchange chromatography, molecular
sieve chromatography, HPLC, native gel electrophoresis

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in combination with gel elution, and preparative
isoelectric focusing ("Isoprime" machine/technique,
Hoefer Scientific). In some cases, two or more of
these techniques may be combined to achieve increased
5    purity.

          If it is anticipated that a CRP1 or B7RP1
polypeptide will be found primarily intracellularly,
the intracellular material (including inclusion
bodies for gram-negative bacteria) can be extracted
10   from the host cell using any standard technique
known to the skilled artisan. For example, the host
cells can be lysed to release the contents of the
periplasm/cytoplasm by French press, homogenization,
and/or sonication followed by centrifugation.

15        If a CRP1 or B7RP1 polypeptide has formed
inclusion bodies in the cytosol, the inclusion
bodies can often bind to the inner and/or outer
cellular membranes and thus will be found primarily
in the pellet material after centrifugation. The
20   pellet material can then be treated at pH extremes
or with chaotropic agent such as a detergent,
guanidine, guanidine derivatives, urea, or urea
derivatives in the presence of a reducing agent such
as dithiothreitol at alkaline pH or tris
25   carboxyethyl phosphine at acid pH  to release, break
apart, and solubilize the inclusion bodies. The
polypeptide in its now soluble form can then be
analyzed using gel electrophoresis,
immunoprecipitation or the like. If it is desired
30   to isolate a CRP1 or B7RP1 polypeptide, isolation
may be accomplished using standard methods such as
those set forth below and in Marston *et al.* (*Meth.
Enz.*, 182:264-275 (1990)). In some cases, a CRP1 or
B7RP1 polypeptide may not be biologically active
35   upon isolation. Various methods for "refolding" or
converting the polypeptide to its tertiary structure

and generating disulfide linkages can be used to restore biological activity. Such methods include contacting the solubilized polypeptide with a solution having a pH usually above 7 and in the

5 presence of a particular concentration of an appropriate chaotrope. In most cases the refolding/oxidation solution will also contain a reducing agent or the reducing agent and the corresponding oxidized form in a specific ratio to

10 generate a particular redox potential allowing for disulfide shuffling to occur in the formation of the protein's cysteine bridge(s). Some of the commonly used redox couples include cysteine/cystamine, glutathione (GSH)/dithiobis GSH, cupric chloride,

15 dithiothreitol(DTT)/dithiane DTT, 2-mercaptoethanol(bME)/dithio-b(ME). In many instances a cosolvent is necessary to increase the efficiency of the refolding and the more common reagents used for this purpose include glycerol,

20 polyethylene glycol of various molecular weights, and arginine.

CRP1 or B7RP1 polypeptides, fragments, and/or derivatives thereof may also be prepared by chemical synthesis methods (such as solid phase

25 peptide synthesis) using techniques known in the art such as those set forth by Merrifield et al., (J. Am. Chem. Soc., 85:2149 (1963)), Houghten et al. (Proc Natl Acad. Sci. USA, 82:5132 (1985)), and Stewart and Young (Solid Phase Peptide Synthesis,

30 Pierce Chemical Co., Rockford, IL (1984)). Such polypeptides may be synthesized with or without a methionine on the amino terminus. Chemically synthesized CRP1 or B7RP1 polypeptides or fragments may be oxidized using methods set forth in these

35 references to form disulfide bridges. CRP1 or B7RP1 polypeptides or fragments are expected to have

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biological activity comparable to CRP1 or B7RP1
polypeptides produced recombinantly or purified from
natural sources, and thus may be used
interchangeably with recombinant or natural CRP1 or
5    B7RP1 polypeptide.


Polypeptides
        The term " CRP1 or B7RP1 polypeptide" refers
to a polypeptide having the amino acid sequence of
10   Figure 1A (SEQ ID NO:2), Figure 2A (SEQ ID NO:7) or
Figure 3A (SEQ ID NO:12) and all related polypeptides
described herein.  Related polypeptides includes
allelic variants, splice variants, fragments,
derivatives, substitution,  deletion, and insertion
15   variants, fusion polypeptides, and orthologs.  Such
related polypeptides may be mature polypeptides, *i.e.*,
polypeptide lacking a signal peptide.  A CRP1 or B7RP1
polypeptide may or may not have amino terminal
methionine, depending on the manner in which they are
20   prepared.
        The term "CRP1 or B7RP1 polypeptide fragment"
refers to a peptide or polypeptide that is less than
the full length amino acid sequence of a CRP1 or B7RP1
polypeptide as set forth in Figure 1A (SEQ ID NO:2),
25   Figure 2A (SEQ ID NO:7) or Figure 3A (SEQ ID NO:12).
Such a fragment may result from truncation at the amino
terminus, truncation at the carboxy terminus, and/or a
deletion internal to the polypeptide sequence.  Such
CRP1 or B7RP1 polypeptides fragments may be prepared
30   with or without an amino terminal methionine.  In
addition, CRP1 or B7RP1 polypeptides fragments may be
naturally-occurring splice variants, other splice
variants, and fragments resulting from naturally
occurring *in vivo* protease activity.  Preferred CRP1
35   or B7RP1 polypeptide fragments include soluble forms of
CRP1 or B7RP1 which lack a functional transmembrane

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domain and comprise part or all of the extracellular
domain of either CRP1 or B7RP1.

The term "CRP1 or B7RP1 polypeptide variants"
refers to CRP1 or B7RP1 polypeptides whose amino acid
5   sequences contain one or more amino acid sequence
substitutions, deletions, and/or additions as compared
to the CRP1 or B7RP1 polypeptides amino acid sequences
set forth in Figure 1A (SEQ ID NO:2), Figure 2A (SEQ ID
NO:7) or Figure 3A (SEQ ID NO:12). Such CRP1 or B7RP1
10  polypeptides variants can be prepared from the
corresponding CRP1 and B7RP1 polypeptides nucleic acid
molecule variants, which have a DNA sequence that
varies accordingly from the DNA sequences for CRP1 or
B7RP1 polypeptides.

15         As used herein, the term "CRP1 or B7RP1
polypeptide derivatives" refers to CRP1 or B7RP1
polypeptides, variants, or fragments thereof, that have
been chemically modified, as for example, by addition
of one or more water soluble polymers, N-linked or
20  O-linked carbohydrates, sugars, phosphates, and/or
other such molecules, where the molecule or molecules
are not naturally attached to wild-type CRP1 or B7RP1
polypeptides. Derivatives further includes deletion of
one or more chemical groups naturally attached to the
25  CRP1 or B7RP1 polypeptide.

As used herein, the terms "biologically
active CRP1 or B7RP1 polypeptides", "biologically
active CRP1 or B7RP1 polypeptide fragments",
"biologically active CRP1 or B7RP1 polypeptide
30  variants", and "biologically active CRP1 or B7RP1
polypeptide derivatives" refer to CRP1 or B7RP1
polypeptides having at least one of the activities
characteristic of CRP1 or B7RP1. One activity is
binding of B7RP1 to CRP1. Another activity is the
35  ability of CRP1 or B7RP1 to stimulate T-cell
proliferation and/or activation.

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The term "ortholog" refers to a polypeptide that corresponds to a polypeptide identified from a species. For example, mouse and human B7RP1 polypeptides are considered orthologs.

5      The term "mature amino acid sequence" refers to a polypeptide lacking a leader sequence.

The term "isolated polypeptide" refers to a polypeptide that is free from at least one contaminating polypeptide that is found in its natural environment, and preferably substantially free from any other contaminating mammalian polypeptides.

The term "identity," as known in the art, is a relationship between the sequences of two or more nucleic acid molecules or two or more polypeptides, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or nucleic acid molecule sequences, as the case may be, as determined by the match between strings of nucleotide or amino acid sequences. "Identity" measures the percent of identical matches between two or more sequences with gap alignments addressed by particular computer programs (i.e., "algorithms").

The term "similarity" refers to a related concept, but in contrast to "identity", a measure of similarity includes both identical matches and conservative substitution matches. Since conservative substitutions apply to polypeptides and not nucleic acid molecules, similarity only deals with polypeptide sequence comparisons. If two polypeptide sequences have, for example, 10/20 identical amino acids, and the remainder are all non-conservative substitutions, then the percent identity and similarity would both be 50%. If in the same example, there are 5 more positions where there are conservative substitutions, then the percent identity remains 50%, but the per cent

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similarity would be 75% (15/20). Therefore, in cases
where there are conservative substitutions, the degree
of similarity between two polypeptide sequences will be
higher than the percent identity between those two
5    sequences. "Conservative" amino acid substitutions are
described herein below in reference to Table I. Based
on Table I, conservative amino acid substitutions are
alternate amino acids selected from the same grouping,
e.g., basic, acidic, uncharged polar, and non-polar.
10   For example, conservative amino acid substitutions for
arginine would be lysine and histidine.
          Identity and similarity can be readily
calculated by known methods, including but not limited
to those described in *Computational Molecular Biology*,
15   Lesk, A.M., ed., Oxford University Press, New York,
1988; *Biocomputing: Informatics and Genome Projects*,
Smith, D.W., ed., Academic Press, New York, 1993;
*Computer Analysis of Sequence Data, Part 1*, Griffin,
A.M., and Griffin, H.G., eds., Humana Press, New
20   Jersey, 1994; *Sequence Analysis in Molecular Biology*,
von Heinje, G., Academic Press, 1987; and *Sequence
Analysis Primer*, Gribskov, M. and Devereux, J., eds.,
M. Stockton Press, New York, 1991; and Carillo, H., and
Lipman, D., *SIAM J. Applied Math.*, 48:1073 (1988).
25             Preferred methods to determine identity
and/or similarity are designed to give the largest
match between the sequences tested. Methods to
determine identity and similarity are codified in
publicly available computer programs. Preferred
30   computer program methods to determine identity and
similarity between two sequences include, but are not
limited to, the GCG program package, including GAP
(Devereux, J., et al., *Nucleic Acids Research* 12(1):387
(1984); Genetics Computer Group, University of
35   Wisconsin, Madison, WI), BLASTP, BLASTN, and FASTA
(Atschul, S.F. et al., *J. Molec. Biol.* 215:403-410

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(1990). The BLAST X program is publicly available from the National Center for Biotechnology Information (NCBI) and other sources (*BLAST Manual*, Altschul, S., et al. NCB NLM NIH Bethesda, MD 20894; Altschul, S., et

5   al., *J. Mol. Biol.* $\underline{215}$:403-410 (1990). The well known Smith Waterman algorithm may also be used to determine identity.

By way of example, using the computer algorithm GAP (Genetics Computer Group, University of

10  Wisconsin, Madison, WI), two polypeptides for which the percent sequence identity is to be determined are aligned for optimal matching of their respective amino acids (the "matched span", as determined by the algorithm). A gap opening penalty (which is calculated

15  as 3 X the average diagonal; the "average diagonal" is the average of the diagonal of the comparison matrix being used; the "diagonal" is the score or number assigned to each perfect amino acid match by the particular comparison matrix) and a gap extension

20  penalty (which is usually 1/10 times the gap opening penalty), as well as a comparison matrix such as PAM 250 or BLOSUM 62 are used in conjunction with the algorithm. A standard comparison matrix (see Dayhoff et al., in: Atlas of Protein Sequence and Structure,

25  vol. 5, supp.3 (1978) for the PAM250 comparison matrix; see Henikoff et al., *Proc. Natl. Acad. Sci USA*, $\underline{89}$:10915-10919 (1992) for the BLOSUM 62 comparison matrix) is also used by the algorithm.

30          Preferred parameters for polypeptide sequence comparison include the following:

Algorithm: Needleman and Wunsch, *J. Mol. Biol.* $\underline{48}$:443-453 (1970)

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Comparison matrix:  BLOSUM 62 from Henikoff and Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915-10919 (1992)

Gap Penalty:  12

5    Gap Length Penalty:  4

Threshold of Similarity: 0

The GAP program is useful with the above parameters.  The aforementioned parameters are the default parameters for polypeptide comparisons (along

10   with no penalty for end gaps) using the GAP algorithm.


Preferred parameters for nucleic acid molecule sequence comparison include the following:


15   Algorithm:  Needleman and Wunsch, *J. Mol Biol.* 48:443-453 (1970)

Comparison matrix:  matches = +10, mismatch = 0

Gap Penalty:  50

Gap Length Penalty:  3

20

The GAP program is also useful with the above parameters.  The aforementioned parameters are the default parameters for nucleic acid molecule comparisons.

25

Other exemplary algorithms, gap opening penalties, gap extension penalties, comparison matrices, thresholds of similarity, etc. may be used by those of skill in the art, including those set forth in

30   the Program Manual, Wisconsin Package, Version 9, September, 1997.  The particular choices to be made will depend on the specific comparison to be made, such as DNA to DNA, protein to protein, protein to DNA; and additionally, whether the comparison is between pairs

35   of sequences (in which case GAP or BestFit are generally preferred) or between one sequence and a

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large database of sequences (in which case FASTA or
BLASTA are preferred).

     Polypeptides that are at least about 70
percent identical will typically have one or more amino
5  acid substitutions, deletions, and/or additions as
compared to a wild type CRP1 or B7RP1 polypeptide. In
preferred embodiment, polypeptides will have about 75%,
80%, 85%, 90% or 95% identity to CRP1 or B7RP1
polypeptides. Usually, the substitutions of the native
10  residue will be either alanine, or a conservative amino
acid so as to have little or no effect on the overall
net charge, polarity, or hydrophobicity of the
polypeptide. Conservative substitutions are set forth
in Table I below.

15

Table I
Conservative Amino Acid Substitutions

| | |
|---|---|
| Basic: | arginine |
| | lysine |
| | histidine |
| Acidic: | glutamic acid |
| | aspartic acid |
| Uncharged Polar: | glutamine |
| | asparagine |
| | serine |
| | threonine |
| | tyrosine |
| Non-Polar: | phenylalanine |
| | tryptophan |
| | cysteine |
| | glycine |
| | alanine |
| | valine |

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                                proline
                                methionine
                                leucine
                                norleucine
                                isoleucine


        CRP1 or B7RP1 polypeptide derivatives are
   provided by the invention.  In one embodiment,
5  chemically modified CRP1 or B7RP1 polypeptide
   compositions in which CRP1 or B7RP1 polypeptides are
   linked to a polymer are included within the scope of
   the present invention.  The polymer selected is
   typically water soluble so that the protein to which
10 it is attached does not precipitate in an aqueous
   environment, such as a physiological environment.
   The polymer selected is usually modified to have a
   single reactive group, such as an active ester for
   acylation or an aldehyde for alkylation, so that the
15 degree of polymerization may be controlled as
   provided for in the present methods.  The polymer
   may be of any molecular weight, and may be branched
   or unbranched.  Included within the scope of the
   invention is a mixture of polymers.  Preferably, for
20 therapeutic use of the end-product preparation, the
   polymer will be pharmaceutically acceptable.
        The water soluble polymer or mixture
   thereof may be selected from the group consisting
   of, for example, polyethylene glycol (PEG),
25 monomethoxy-polyethylene glycol, dextran, cellulose,
   or other carbohydrate based polymers, poly-(N-vinyl
   pyrrolidone) polyethylene glycol, propylene glycol
   homopolymers, a polypropylene oxide/ethylene oxide
   co-polymer, polyoxyethylated polyols (e.g.,
30 glycerol) and polyvinyl alcohol.

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For the acylation reactions, the polymer(s)
selected should have a single reactive ester group.
For reductive alkylation, the polymer(s) selected
should have a single reactive aldehyde group.  A
5    preferred reactive aldehyde is polyethylene glycol
propionaldehyde, which is water stable, or mono C1-C10
alkoxy or aryloxy derivatives thereof (see U.S. Patent
No. 5,252,714).

Pegylation of CRP1 or B7RP1 polypeptides may
10   be carried out by any of the pegylation reactions known
in the art, as described for example in the following
references: *Focus on Growth Factors* 3: 4-10 (1992); EP
0 154 316; and EP 0 401 384.  Preferably, the
pegylation is carried out via an acylation reaction or
15   an alkylation reaction with a reactive polyethylene
glycol molecule (or an analogous reactive water-soluble
polymer) as described below.

A particularly preferred water-soluble
polymer for use herein is polyethylene glycol,
20   abbreviated PEG.  As used herein, polyethylene glycol
is meant to encompass any of the forms of PEG that have
been used to derivatize other proteins, such as mono-
(C1-C10) alkoxy- or aryloxy-polyethylene glycol.

In general, chemical derivatization may be
25   performed under any suitable conditions used to react a
biologically active substance with an activated polymer
molecule.  Methods for preparing pegylated CRP1 and
B7RP1 polypeptides will generally comprise the steps of
(a) reacting the polypeptide with polyethylene glycol
30   (such as a reactive ester or aldehyde derivative of
PEG) under conditions whereby CRP1 or B7RP1 polypeptide
becomes attached to one or more PEG groups, and (b)
obtaining the reaction product(s).  In general, the
optimal reaction conditions for the acylation reactions
35   will be determined based on known parameters and the
desired result.  For example, the larger the ratio of

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PEG: protein, the greater the percentage of poly-
pegylated product.

Generally, conditions which may be alleviated
or modulated by administration of CRP1 or B7RP1 polymer
conjugates include those described herein for non-
conjugated CRP1 or B7RP1 polypeptides. However, the
conjugated disclosed herein may have additional
activities, enhanced or reduced biological activity, or
other characteristics, such as increased or decreased
half-life, as compared to the non-derivatized
molecules.

CRP1 or B7RP1 polypeptides, fragments
variants, and derivatives, may be employed alone,
together, or in combination with other pharmaceutical
compositions. CRP1 or B7RP1 polypeptides, fragments,
variants, and derivatives may be used in combination
with cytokines, growth factors, antibiotics, anti-
inflammatories, and/or chemotherapeutic agents as is
appropriate for the indication being treated.

The invention provides for selective binding
agents of CRP1 or B7RP1. A selective binding agent
refers to a molecule having specificity for CRP1 or
B7RP1 and may include a protein, peptide, nucleic acid,
carbohydrate, lipid or small molecular weight compound.
A selective binding agent interacts either with CRP1 or
B7RP1 and in turn regulates the binding of CRP1 to
B7RP1. In one embodiment, a selective binding agent
partially or completely blocks the binding of CRP1 to
B7RP1 and partially or completely inhibits at least one
biological activity of CRP-1 or B7RP-1, such as immune
costimulatory activity. In another embodiment, the
selective binding agent is an antibody. The antibody
may be immunoreactive with either CRP1 or B7RP1 and is
preferably immunoreactive with B7RP1. In yet another
embodiment of the invention, an antibody reactive with

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B7RP1 binds to an eptiope on B7RP1 such that binding to CRP1 is partially or completely blocked and at least one biological activity of B7RP1, such as immune costimulatory activity, is partially or completely
5    inhibited.  The term partially inhibited means that at least a detectable level of inhibition has occurred. The term completely inhibited means that no further increase in inhibition has occurred.

CRP1 or B7RP1 polypeptides, fragments,
10   variants, and/or derivatives may be used to prepare antibodies using methods known in the art. ·Thus, antibodies that react with the CRP1 or B7RP1 polypeptides, as well as reactive fragments of such antibodies, are also contemplated as within the scope
15   of the present invention.  The antibodies may be polyclonal, monoclonal, recombinant, chimeric, single-chain and/or bispecific.  Typically, the antibody or fragment thereof will either be of human origin, or will be "humanized", *i.e.*, prepared so as to prevent or
20   minimize an immune reaction to the antibody when administered to a patient.  The antibody fragment may be any fragment that is reactive with CRP1 and B7RP1 polypeptides of the present invention, such as, Fab, Fab', etc.  Also provided by this invention are the
25   hybridomas generated by presenting any CRP1 or B7RP1 polypeptide or fragments thereof as an antigen to a selected mammal, followed by fusing cells (*e.g.*, spleen cells) of the mammal with certain cancer cells to create immortalized cell lines by known techniques.
30   The methods employed to generate such cell lines and antibodies directed against all or portions of a human CRP1 or B7RP1 polypeptide of the present invention are also encompassed by this invention.

Monoclonal antibodies of the invention
35   include "chimeric" antibodies in which a portion of the

heavy and/or light chain is identical with or homologous to corresponding sequence in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the
5    remainder of the chains(s) is identical with or homologous to corresponding sequence in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired
10   biological activity (U.S. Patent No. 4,816,567; Morrison, et al., Proc. Natl. Acad. Sci. 81, 6851-6855 (1985)).

In a preferred embodiment, the chimeric anti-CRP1 or B7RP1 antibody is a "humanized" antibody.
15   Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. Humanization can be performed following methods known in the art (Jones, et
20   al., Nature 321, 522-525 (1986); Riechmann, et al., Nature, 332, 323-327 (1988); Verhoeyen, et al., Science 239, 1534-1536 (1988)), by substituting rodent complementarily-determining regions (CDRs) for the corresponding regions of a human antibody.

25   Also encompassed by the invention are fully human anti-CRP1 or anti-B7RP1 antibodies. Such antibodies may be produced by immunization with a CRP1 or B7RP1 antigen of transgenic animals (e.g., mice) that are capable of producing a repertoire of human
30   antibodies in the absence of endogenous immunoglobulin production. See, for example, Jakobovits, et al., Proc. Natl. Acad. Sci. 90, 2551-2555 (1993); Jakobovits, et al., Nature 362, 255-258 (1993). Human antibodies can also be produced in phage-display
35   libraries (Hoogenboom, et al., J. Mol. Biol. 227, 381 (1991); Marks, et al., J. Mol. Biol. 222, 581 (1991).

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Selective binding agents of the invention may
be used to regulate the binding of CRP1 to B7RP1 and
regulate at least one biological activity mediated by
CRP1 and B7RP1 such as immune co-stimulation.  An
5    example of such selective binding agents are antibodies
immunoreactive with either CRP1 or B7RP1.  The
antibodies may be used therapeutically, such as to
inhibit binding of the CRP1 and B7RP1 polypeptide to
its binding partner.  The antibodies may further be
10   used for *in vivo* and *in vitro* diagnostic purposes, such
as in labeled form to detect the presence of CRP1 and
B7RP1 polypeptide in a body fluid or cell sample.

Pharmaceutical Compositions and Administration
15         Pharmaceutical compositions of CRP1 or B7RP1
polypeptides are within the scope of the present
invention.  Such compositions may comprise a
therapeutically effective amount of the polypeptide or
fragments, variants, or derivatives in admixture with a
20   pharmaceutically acceptable carrier.  In preferred
embodiments, pharmaceutical compositions comprise CRP1
or B7RP1 polypeptides as soluble forms which comprise
part or all of a CRP1 or B7RP1 extracellular domain.
Typically, a CRP1 and B7RP1 polypeptide therapeutic
25   compound will be administered in the form of a
composition comprising purified polypeptide, fragment,
variant, or derivative in conjunction with one or more
physiologically acceptable carriers, excipients, or
diluents.  The carrier material may be water for
30   injection, preferably supplemented with other materials
common in solutions for administration to mammals.
Neutral buffered saline or saline mixed with serum
albumin are exemplary appropriate carriers.
Preferably, the product is formulated as a lyophilizate
35   using appropriate excipients (*e.g.*, sucrose).  Other
standard carriers, diluents, and excipients may be

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included as desired. Other exemplary compositions
comprise Tris buffer of about pH 7.0-8.5, or acetate
buffer of about pH 4.0-5.5, which may further include
sorbitol or a suitable substitute therefor.

5    CRP1 or B7RP1 pharmaceutical compositions can
be administered parenterally. Alternatively, the
compositions may be administered intravenously or
subcutaneously. When systemically administered, the
therapeutic compositions for use in this invention may

10   be in the form of a pyrogen-free, parenterally
acceptable aqueous solution. The preparation of such
pharmaceutically acceptable protein solutions, with due
regard to pH, isotonicity, stability and the like, is
within the skill of the art.

15   Therapeutic formulations of CRP1 and B7RP1
polypeptide compositions useful for practicing the
present invention may be prepared for storage by mixing
the selected composition having the desired degree of
purity with optional physiologically acceptable

20   carriers, excipients, or stabilizers (Remington's
Pharmaceutical Sciences, 18th Edition, A.R. Gennaro,
ed., Mack Publishing Company (1990)) in the form of a
lyophilized cake or an aqueous solution. Acceptable
carriers, excipients or stabilizers are nontoxic to

25   recipients and are preferably inert at the dosages and
concentrations employed, and include buffers such as
phosphate, citrate, or other organic acids;
antioxidants such as ascorbic acid; low molecular
weight polypeptides; proteins, such as serum albumin,

30   gelatin, or immunoglobulins; hydrophilic polymers such
as polyvinylpyrrolidone; amino acids such as glycine,
glutamine, asparagine, arginine or lysine;
monosaccharides, disaccharides, and other carbohydrates
including glucose, mannose, or dextrins; chelating

35   agents such as EDTA; sugar alcohols such as mannitol or
sorbitol; salt-forming counterions such as sodium;

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and/or nonionic surfactants such as Tween, pluronics or polyethylene glycol (PEG).

An effective amount of a CRP1 or B7RP1 polypeptide composition(s) to be employed
5   therapeutically will depend, for example, upon the therapeutic objectives such as the indication for which the CRP1 and B7RP1 polypeptide is being used, the route of administration, and the condition of the patient. Accordingly, it will be necessary for the therapist to
10   titer the dosage and modify the route of administration as required to obtain the optimal therapeutic effect. A typical daily dosage may range from about 0.1 µg/kg to up to 100 mg/kg or more, depending on the factors mentioned above. Typically, a clinician will
15   administer the composition until a dosage is reached that achieves the desired effect. The composition may therefore be administered as a single dose, or as two or more doses (which may or may not contain the same amount of a CRP1 or B7RP1 polypeptide) over time, or as
20   a continuous infusion via implantation device or catheter.

As further studies are conducted, information will emerge regarding appropriate dosage levels for treatment of various conditions in various patients,
25   and the ordinary skilled worker, considering the therapeutic context, the type of disorder under treatment, the age and general health of the recipient, will be able to ascertain proper dosing.

The CRP1 or B7RP1 polypeptide composition to
30   be used for in vivo administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes. Where the composition is lyophilized, sterilization using these methods may be conducted either prior to, or following, lyophilization
35   and reconstitution. The composition for parenteral

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administration ordinarily will be stored in lyophilized
form or in solution.

Therapeutic compositions generally are placed
into a container having a sterile access port, for
5    example, an intravenous solution bag or vial having a
stopper pierceable by a hypodermic injection needle.

The route of administration of the
composition is in accord with known methods, *e.g.* oral,
injection or infusion by intravenous, intraperitoneal,
10   intracerebral (intraparenchymal),
intracerebroventricular, intramuscular, intraocular,
intraarterial, or intralesional routes, or by sustained
release systems or implantation device which may
optionally involve the use of a catheter.  Where
15   desired, the compositions may be administered
continuously by infusion, bolus injection or by
implantation device.

Alternatively or additionally, the
composition may be administered locally via
20   implantation into the affected area of a membrane,
sponge, or other appropriate material on to which CRP1
and B7RP1 polypeptide has been absorbed.

Where an implantation device is used, the
device may be implanted into any suitable tissue or
25   organ, and delivery of a CRP1 or B7RP1 polypeptide may
be directly through the device via bolus, or via
continuous administration, or via catheter using
continuous infusion.  A CRP1 or B7RP1 polypeptide may
be administered in a sustained release formulation or
30   preparation.  Suitable examples of sustained-release
preparations include semipermeable polymer matrices in
the form of shaped articles, *e.g.* films, or
microcapsules.  Sustained release matrices include
polyesters, hydrogels, polylactides (U.S. 3,773,919, EP
35   58,481), copolymers of L-glutamic acid and gamma ethyl-
L-glutamate (Sidman *et al, Biopolymers,* <u>22</u>: 547-556

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(1983)), poly (2-hydroxyethyl-methacrylate) (Langer *et al.*, *J. Biomed. Mater. Res.*, 15: 167-277 (1981)] and Langer, *Chem. Tech.*, 12: 98-105 (1982)), ethylene vinyl acetate (Langer *et al.*, *supra*) or poly-D(-)-3-
5   hydroxybutyric acid (EP 133,988). Sustained-release compositions also may include liposomes, which can be prepared by any of several methods known in the art (*e.g.*, Eppstein *et al.*, *Proc. Natl. Acad. Sci. USA*, 82: 3688-3692 (1985); EP 36,676; EP 88,046; EP 143,949).
10

In some cases, it may be desirable to use CRP1 or B7RP1 polypeptide compositions in an *ex vivo* manner. Here, cells, tissues, or organs that have been removed from the patient are exposed to a CRP1 or B7RP1
15  polypeptide compositions after which the cells, tissues and/or organs are subsequently implanted back into the patient.

In other cases, a CRP1 or B7RP1 polypeptide may be delivered through implanting into patients
20  certain cells that have been genetically engineered, using methods such as those described herein, to express and secrete the polypeptides, fragments, variants, or derivatives. Such cells may be animal or human cells, and may be derived from the patient's own
25  tissue or from another source, either human or non-human. Optionally, the cells may be immortalized. However, in order to decrease the chance of an immunological response, it is preferred that the cells be encapsulated to avoid infiltration of surrounding
30  tissues. The encapsulation materials are typically biocompatible, semi-permeable polymeric enclosures or membranes that allow release of the protein product(s) but prevent destruction of the cells by the patient's immune system or by other detrimental factors from the
35  surrounding tissues.

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        Methods used for membrane encapsulation of
cells are familiar to the skilled artisan, and
preparation of encapsulated cells and their
implantation in patients may be accomplished without
5    undue experimentation.  See, *e.g.*, U.S Patent Nos.
4,892,538; 5,011,472; and 5,106,627.  A system for
encapsulating living cells is described in PCT WO
91/10425 (Aebischer et al.).  Techniques for
formulating a variety of other sustained or controlled
10   delivery means, such as liposome carriers, bio-erodible
particles or beads, are also known to those in the art,
and are described, for example, in U.S. Patent No.
5,653,975.  The cells, with or without encapsulation,
may be implanted into suitable body tissues or organs
15   of the patient.

        As discussed above, it may be desirable to
treat cell preparations with one or more CRP1 or B7RP1
polypeptides, variants, derivatives and/or fragments.
This can be accomplished by exposing, for example,
20   cells comprising T-cells, such as bone marrow cells, to
the polypeptide, variant, derivative, or fragment
directly, where it is in a form that is permeable to
the cell membrane.  For example, cells comprising
T-cells may be exposed to a B7RP1 polypeptide in order
25   to activate T-cell function and the cells so treated
are implanted in the patient.

        Alternatively, gene therapy can be employed.
One manner in which gene therapy can be applied is to
use a CRP1 or B7RP1 gene (either genomic DNA, cDNA,
30   and/or synthetic DNA encoding a CRP1 or B7RP1
polypeptide, or a fragment, variant, or derivative
thereof) which may be operably linked to a constitutive
or inducible promoter to form a "gene therapy DNA
construct".  The promoter may be homologous or
35   heterologous to the endogenous CRP1 or B7RP1 gene,
provided that it is active in the cell or tissue type

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into which the construct will be inserted.  Other
components of the gene therapy DNA construct may
optionally include, as required, DNA molecules designed
for site-specific integration (e.g., endogenous
5     flanking sequences useful for homologous
recombination), tissue-specific promoter, enhancer(s)
or silencer(s), DNA molecules capable of providing a
selective advantage over the parent cell, DNA molecules
useful as labels to identify transformed cells,
10    negative selection systems, cell specific binding
agents (as, for example, for cell targeting) cell-
specific internalization factors, and transcription
factors to enhance expression by a vector as well as
factors to enable vector manufacture.
15          This gene therapy DNA construct can then be
introduced into the patient's cells (either ex vivo or
in vivo).  One means for introducing the gene therapy
DNA construct is via viral vectors.  Suitable viral
vectors typically used in gene therapy for delivery of
20    gene therapy DNA constructs include, without
limitation, adenovirus, adeno-associated virus, herpes
simplex virus, lentivirus, papilloma virus, and
retrovirus vectors.  Some of these vectors, such as
retroviral vectors, will deliver the gene therapy DNA
25    construct to the chromosomal DNA of the patient's
cells, and the gene therapy DNA construct can integrate
into the chromosomal DNA; other vectors will function
as episomes and the gene therapy DNA construct will
remain in the cytoplasm.  The use of gene therapy
30    vectors is described, for example, in U.S. Patent Nos.
5,672,344, 5,399,346.
            Alternative means to deliver gene therapy DNA
constructs to a patient's cells without the use of
viral vectors include, without limitation, liposome-
35    mediated transfer, direct injection of naked DNA,
receptor-mediated transfer (ligand-DNA complex),

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electroporation, calcium phosphate precipitation, and microparticle bombardment (e.g., "gene gun"). See U.S. Patent Nos. 4,970,154, WO 96/40958, 5,679,559, 5,676,954, and 5,593,875.

5      Another means to increase endogenous CRP1 or B7RP1 polypeptide expression in a cell via gene therapy is to insert one or more enhancer elements into the CRP1 or B7RP1 polypeptide promoter, where the enhancer element(s) can serve to increase transcriptional

10     activity of a CRP1 or B7RP1 polypeptide gene. The enhancer element(s) used will be selected based on the tissue in which one desires to activate the gene(s); enhancer elements known to confer promoter activation in that tissue will be selected. For example, if a

15     CRP1 or B7RP1 polypeptide is to be "turned on" in T-cells, the *lck* promoter enhancer element may be used. Here, the functional portion of the transcriptional element to be added may be inserted into a fragment of DNA containing a CRP1 or B7RP1 polypeptide promoter

20     (and optionally, vector, 5' and/or 3' flanking sequence, etc.) using standard cloning techniques. This construct, known as a "homologous recombination construct" can then be introduced into the desired cells either *ex vivo* or *in vivo*.

25     Gene therapy can be used to decrease CRP1 or B7RP1 polypeptide expression by modifying the nucleotide sequence of the endogenous promoter(s). Such modification is typically accomplished via homologous recombination methods. For example, a DNA

30     molecule containing all or a portion of the promoter of a CRP1 or B7RP1 gene(s) selected for inactivation can be engineered to remove and/or replace pieces of the promoter that regulate transcription. Here, the TATA box and/or the binding site of a transcriptional

35     activator of the promoter may be deleted using standard molecular biology techniques; such deletion can inhibit

promoter activity thereby repressing transcription of
the corresponding CRP1 or B7RP1 gene.  Deletion of the
TATA box or transcription activator binding site in the
promoter may be accomplished by generating a DNA

5    construct comprising all or the relevant portion of a
CRP1 or B7RP1 polypeptide promoter(s) (from the same or
a related species as a CRP1 or B7RP1 gene(s) to be
regulated) in which one or more of the TATA box and/or
transcriptional activator binding site nucleotides are

10   mutated via substitution, deletion and/or insertion of
one or more nucleotides such that the TATA box and/or
activator binding site has decreased activity or is
rendered completely inactive.  This construct, which
also will typically contain at least about 500 bases of

15   DNA that correspond to the native (endogenous) 5' and
3' flanking regions of the promoter segment that has
been modified, may be introduced into the appropriate
cells (either *ex vivo* or *in vivo*) either directly or
via a viral vector as described above.  Typically,

20   integration of the construct into the genomic DNA of
the cells will be via homologous recombination, where
the 5' and 3' flanking DNA sequences in the promoter
construct can serve to help integrate the modified
promoter region via hybridization to the endogenous

25   chromosomal DNA.
        Other gene therapy methods may also be
employed where it is desirable to inhibit one or more
CRP1 or B7RP1 polypeptides.  For example, antisense DNA
or RNA molecules, which have a sequence that is

30   complementary to at least a portion of a selected CRP1
or B7RP1 polypeptide gene(s) can be introduced into the
cell.  Typically, each such antisense molecule will be
complementary to the start site (5' end) of each
selected CRP1 or B7RP1 gene.  When the antisense

35   molecule then hybridizes to the corresponding CRP1 or

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B7RP1 polypeptide mRNA, translation of this mRNA is prevented.

Alternatively, gene therapy may be employed to create a dominant-negative inhibitor of one or more CRP1 or B7RP1 polypeptides. In this situation, the DNA encoding a mutant full length or truncated polypeptide of each selected CRP1 or B7RP1 polypeptide can be prepared and introduced into the cells of a patient using either viral or non-viral methods as described above. Each such mutant is typically designed to compete with endogenous polypeptide in its biological role.

Agonists and Antagonists

The invention also provides for agonists and antagonists of CRP1 or B7RP1 which regulate the activity of either or both molecules. Agonists and antagonists may be identified from test molecules which alter the binding of B7RP1 to CRP1.

The term "test molecule(s)" refers to the molecule(s) that is/are under evaluation for the ability to bind a CRP1 or B7RP1 polypeptide and thereby alter the binding of B7RP1 to CRP1. Preferably, the test molecule will bind with an affinity constant of at least about $10^6$M.

A variety of assays may be used to measure binding of B7RP1 to CRP1. These assays may be used to screen test molecules for their ability to increase or decrease the rate or extent of binding of B7RP1 to CRP1. In one type of assay, a CRP1 polypeptide, preferably a soluble form of CRP1 such as an extracellular domain, is immobilized by attachment to the bottom of the wells of a microtiter plate. Radiolabeled B7RP1 and the test molecule(s) can then be added either one at a time (in either order) or simultaneously to the wells. After incubation, the

wells can be washed and counted using a scintillation counter for radioactivity to determine the extent of binding to CRP1 protein by B7RP1. Typically, the molecules will be tested over a range of

5   concentrations, and a series of control wells lacking one or more elements of the test assays can be used for accuracy in evaluation of the results. An alternative to this method involves reversing the "positions" of the proteins, i.e., immobilizing B7RP1 to the

10  microtiter plate wells, incubating with the test molecule and radiolabeled CRP1, and determining the extent of CRP1 binding (see, for example, chapter 18 of Current Protocols in Molecular Biology, Ausubel et al., eds., John Wiley & Sons, New York, NY [1995]).

15          As an alternative to radiolabelling, CRP1 or B7RP1 may be conjugated to biotin and the presence of biotinylated protein can then be detected using streptavidin linked to an enzyme, such as horse radish peroxidase [HRP] or alkaline phosphatase [AP], that can

20  be detected colorometrically, or by fluorescent tagging of streptavidin. An antibody directed to CRP1 or B7RP1 that is conjugated to biotin may also be used and can be detected after incubation with enzyme-linked streptavidin linked to AP or HRP

25          CRP1 and B7RP1 may also be immobilized by attachment to agarose beads, acrylic beads or other types of such inert substrates. The substrate-protein complex can be placed in a solution containing the complementary protein and the test compound; after

30  incubation, the beads can be precipitated by centrifugation, and the amount of binding between CRP1 and B7RP1 can be assessed using the methods described above. Alternatively, the substrate-protein complex can be immobilized in a column and the test molecule

35  and complementary protein passed over the column. Formation of a complex between CRP1 and B7RP1 can then

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be assessed using any of the techniques set forth above, *i.e.*, radiolabelling, antibody binding, or the like.

Another type of *in vitro* assay that is useful for identifying a test molecule which increases or decreases formation of an CRP1/B7RP1 complex is a surface plasmon resonance detector system such as the Biacore assay system (Pharmacia, Piscataway, NJ). The Biacore system may be carried out using the manufacturer's protocol. This assay essentially involves covalent binding of either CRP1 or B7RP1 to a dextran-coated sensor chip which is located in a detector. The test compound and the other complementary protein can then be injected into the chamber containing the sensor chip either simultaneously or sequentially and the amount of complementary protein that binds can be assessed based on the change in molecular mass which is physically associated with the dextran-coated side of the of the sensor chip; the change in molecular mass can be measured by the detector system.

In some cases, it may be desirable to evaluate two or more test compounds together for use in increasing or decreasing formation of a CRP1/B7RP1 complex. In these cases, the assays set forth above can be readily modified by adding such additional test compound(s) either simultaneously with, or subsequently to, the first test compound. The remainder of steps in the assay are as set forth above.

*In vitro* assays such as those described above may be used advantageously to screen rapidly large numbers of compounds for effects on complex formation by CRP1 and B7RP1. The assays may be automated to screen compounds generated in phage display, synthetic peptide and chemical synthesis libraries.

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Compounds which increase or decrease complex formation of CRP1 and B7RP1 may also be screened in cell culture using cells and cell lines expressing either polypeptide. Cells and cell lines may be
5    obtained from any mammal, but preferably will be from human or other primate, canine, or rodent sources. The binding of B7RP1 to cells expressing CRP1 on the surface is evaluated in the presence or absence of test molecules and the extent of binding may be determined
10   by, for example, flow cytometry using a biotinylated antibody to B7RP1. Cell culture assays may be used advantageously to further evaluate compounds that score positive in protein binding assays described above.

15   Therapeutic Uses

Polypeptides of the invention, and agonists and antagonists thereof, may be used to regulate T-cell function. Agonists and antagonists include those molecules which regulate CRP1 and/or B7RP1 activity and
20   either increase or decrease at least one activity of a CRP1 or B7RP1 protein such as one activity associated with T-cell functions, for example, T-cell activation. Agonists or antagonists may be co-factors, such as a protein, peptide, carbohydrate, lipid, or small
25   molecular weight molecule, which interact with either CRP1 or B7RP1 and thereby regulate their activity. Potential polypeptide agonists or antagonists include antibodies that react with either soluble or membrane-bound forms of CRP1 or B7RP1 which comprise part or all
30   of the extracellular domains of the said proteins. Molecules that regulate CRP1 or B7RP1 expression typically include nucleic acids encoding CRP1 or B7RP1 protein that can act as anti-sense regulators of expression.
35   CRP1 or B7RP1 polypeptides, and agonists and antagonists thereof, may be used in the treatment of

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autoimmune disease, graft survival, immune cell
activation for inhibiting tumor cell growth, T-cell
dependent B-cell mediated diseases, and cancer gene
immunotherapy.  In one embodiment, antagonists or
5   inhibitors of CRP1 and/or B7RP1 function may be
beneficial to alleviate symptoms in diseases with
chronic immune cell dysfunction.  Autoimmune diseases,
such as systemic lupus erythematosis, rheumatoid
arthritis, immune thrombocytopenic purpura (ITP), and
10  psoriasis, may be treated with antagonists or
inhibitors of CRP-1/B7RP-1.  In addition, chronic
inflammatory diseases, such as inflammatory bowel
disease (Crohn's disease and ulcerative colitis),
Grave's disease, Hashimoto's thyroiditis, and diabetes
15  mellitis, may also be treated with inhibitors to CRP-
1/B7RP-1.  As described in Example 18, CRP-1-Fc
inhibits and B7RP-1-Fc enhances, the onset of disease
in a rodent rheumatoid arthritis disease model. These
opposite effects in this model support an agonistic
20  role for the B7RP-1-Fc protein and an antagonistic role
for the CRP-1-Fc protein. The results also illustrate
how T-cell responses can be regulated by manipulation
of this pathway and the significance of this pathway in
the progression of rheumatoid arthritis. In addition,
25  as described in Example 19, expression of B7RP-1-Fc *in
vivo* stimulates an inflammatory bowel disease (IBD)
phenotype in transgenic mice. This example supports the
role for B7RP-1/CRP-1 in the development of
inflammation in the intestine. Therefore, antagonists
30  of the B7RP-1/CRP-1 pathway may be used to treat human
IBD.
        Antagonists of CRP1 or B7RP1 may be used as
immunosuppressive agents for bone marrow and organ
transplantation and may be used to prolong graft
35  survival.  Such antagonists may provide significant
advantages over existing treatment.  Bone marrow and

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organ transplantation therapy must contend with T-cell
mediated rejection of the foreign cells or tissue by
the host.  Present therapeutic regimens for inhibiting
T-cell mediated rejection involve treatment with the
5   drugs cyclosporine or FK506.  While drugs are
effective, patients suffer from serious side effects,
including hepatotoxicity, nephrotoxicity, and
neurotoxicity. The target for the cyclosporin/FK506
class of therapeutics is calcineurin, a phosphatase
10  with ubiquitous expression. Since CRP1 expression is
restricted to T-cells, inhibitors of CRP1 or B7RP1 may
lack the severe side effects observed with the use of
the present immunotherapeutic agents.
       Antagonists of CRP1 or B7RP1 may be used as
15  immunosuppressive agents for autoimmune disorders, such
as rheumatoid arthritis, psoriasis, multiple sclerosis,
diabetes, and systemic lupus erythematosus.
       Antagonists of the CRP1/B7RP1-mediated
costimulatory pathway may also be used to alleviate
20  toxic shock syndrome, inflammatory bowel disease,
allosensitization due to blood transfusions, T-cell
dependent B-cell mediated diseases, and the treatment
of graft vs. host disease.
       Antibodies, soluble proteins comprising for
25  example extracellular domains, and other regulators of
CRP1 or B7RP1 that result in prolonged or enhanced T-
cell activation can be used to increased the immune
response to tumors.  Example 20 shows B7RP-1-Fc can
inhibit tumor cell growth in mice. Similarly, human
30  B7RP-1-Fc, or other activators of the B7RP-1/CRP-1
pathway, may be used to enhance immune responses
against human tumors. Anti-tumor activity is generally
considered to have a strong cytolytic T-lymphocyte
component. In fact, the anti-tumor effects of B7-Fc
35  fusion proteins (Sturmhoefel et al., Cancer Res. 59:
4964-4972, 1999) were mediated by cytolytic CD8+ T-

cells. Since CRP-1 is also expressed on cytolytic CD8+
T-cells (Example 9), it is probable that the anti-tumor
effects demonstrated in Example 20 were due to B7RP-1-
Fc action on CD8+ cells. The B7RP-1/CRP-1 pathway can

5   also be manipulated to regulate CTL response in a
number of other clinical settings, including allograft
transplantation, graft vs. host disease, and autoimmune
diseases.

Gene therapy using B7RP1 genes of the

10  invention may be used in cancer immunotherapy. B7RP1
genes introduced into cancer cells can transform them
into antigen presenting cells that can be recognized by
the T-cells of the immune system when introduced back
into an animal. Recognition of the transfected tumor

15  cells by the T-cells results in eradication of both
tumors cells expressing, or not expressing, the B7RP1
gene. This immunotherapy approach may be used for
various leukemias, sarcomas, melanomas,
adenocarcinomas, breast carcinomas, prostate tumors,

20  lung carcinomas, colon carcinomas and other tumors.
This invention encompasses using the B7RP1 gene in a
similar manner to enhance T-cell activation in response
to variety of tumors.

As described in Example 14, the phenotype of

25  transgenic mice expressing B7RP1 indicates that B7RP1
is important in the control of antibody production.
Agonists and antagonists of B7RP1 protein activity may
be useful in therapeutic indications that call for the
inhibition or enhancement of antibody production.

30       For instance, many vaccines act by eliciting
an effective and specific antibody response. Some
vaccines, especially those against intestinal micro-
organisms (e.g. Hepatitis A virus, and Salmonellas),
elicit a short-lived antibody response. It is desirable

35  to potentiate and prolong this response in order to
increase the effectiveness of the vaccine. Therefore,

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soluble B7RP1 or activating antibodies to CRP1 may
serve as a vaccine adjuvant.

　　　　Anti-viral responses may also be enhanced by
activators or agonists of the B7RP-1/CRP-1 pathway. The
5    data in Example 20 indicate that cellular immunity is
enhanced by B7RP-1-Fc. The enhancement of cellular
immune functions by B7RP-1-Fc, or other activators of
the B7RP-1/CRP-1 pathway, may also be beneficial in
eliminating virus-infected cells. In a complementary
10   fashion, B7RP-1-Fc has effects on humoral immune
functions that may enhance antibody mediated responses
as observed in Example 13 that may function to help
clear free-virus from the body.

　　　　Conversely, there are a number of clinical
15   conditions that would be ameliorated by the inhibition
of antibody production. Hypersensitivity is a normally
beneficial immune response that is exaggerated or
inappropriate, and leads to inflammatory reactions and
tissue damage. Hypersensitivity reactions which are
20   antibody-mediated may be particularly susceptible to
antagonism by inhibitors of B7RP1 activity. Allergies,
hay fever, asthma, and acute edema cause type I
hypersensitivity reactions, and these reactions may be
suppressed by protein, antibody or small molecule
25   inhibitors of B7RP1 activity.

　　　　Diseases that cause antibody-mediated
hypersensitivity reactions, including systemic lupus
erythematosis, arthritis (rheumatoid arthritis,
reactive arthritis, psoriatic arthritis), nephropathies
30   (glomerulo-nephritis, membranous, mesangiocapillary,
focal segmental, focal necrotizing, crescentic,
proliferative - tubulopathies), skin disorders
(pemphigus and pemphigoid, erythema nodosum),
endocrinopathies (thyroiditis - Grave's, Hashimoto's -
35   insulin dependent diabetes mellitus), various
pneumopathies (especially extrinsic alveolitis),

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various vasculopathies, coeliac disease, with aberrant
production of IgA, many anemias and thrombocytopenias,
Guillain-Barre Syndrome, and myasthenia gravis, may be
treated with B7RP1 antagonists.

5          In addition, lymphoproliferative disorders,
such as multiple myeloma, Waldenstrom's
macroglobulinemia, and crioglobulinemias, may be
inhibited by protein, antibody, or small molecule
antagonists of B7RP1.

10          Finally, graft versus host disease, an
"artificial" immune disorder, may benefit from the
inhibition of antibody production by B7RP1 antagonists.


          The following examples are offered to more
15   fully illustrate the invention, but are not construed
as limiting the scope thereof.



                        Example 1
20              CRP1 cDNA and Amino Acid Sequence


          Female C57/ Black 6 mice were sacrificed, and
the small intestines were excised, and the Peyer's
patches were removed.  The small intestine tissue was
25   sliced open and washed to remove mucus and other
debris.  The epithelial layer, which contains the
intestinal intraepithelial cells (iIELs), was released
by gentle agitation in RPMI-1640 supplemented with 1 mM
dithiothreitol (DTT), for 20 minutes at 37°C.
30   Disassociated cells were passed through a 100 μ filter,
washed in 50 ml of RPMI-1640, mixed to further break up
clumps of cells, and then passed through a 40 μ
strainer to obtain single cell populations.  These
cells were then washed again in a 50 ml volume of RPMI-
35   1640 to ensure the removal of the residual DTT.  The
tissue was then agitated and washed as before to gather

the remaining iIELs. The iIELs were separated from the
adipose cells and most epithelial cells on a 3-step
Percol gradient, with the iIELs banding at the 40% to
80% interface. These cells were then washed twice with
5    RPMI-1640 to remove traces of Percol, immunostained
with CD103 (integrin alpha IEL) antibodies, and
separated on a FACs Star cell sorter. These sorted
cells were then either used to prepare total RNA
directly using Trizol (Gibco BRL, Gaithersburg, MD), or
10   activated overnight on plate-bound activating
antibodies, which crosslink the gamma/delta TCR,
alpha/beta TCR, or CD3. The RNA was prepared as above
and pooled for use in constructing EST cDNA libraries.

       A cDNA clone, designated smi12-00082-a1,
15   contained nucleotide sequence homology to CD28 (Figure
1B). Translation of the sequence and subsequent
comparison to known proteins in a public database
revealed 19% amino acid identity with murine CD28
(Figure 1B). This low homology was significant because
20   murine CD28 shares only 26% amino acid identity with
murine CTLA-4. All of the putative cysteines thought
to be critical for intra- and inter-molecular cysteine
bonding in the CD28/CTLA-4 family were found to be
conserved (amino acid residues 83, 109, and 137;
25   relative to the initiating methionine). In addition,
the overall length of the putative open reading frame,
and the relative position of the transmembrane domain,
were similar to those of both CD28 and CTLA-4. We
named the gene CRP1, for CD28-Related Protein-1.

30

Example 2
Cloning of Human CRP1 cDNA

       The nucleic acid sequence encoding human CRP1
35   protein is identified by the following procedures. A
human cDNA library was prepared from enriched

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lymphocytes from peripheral human blood from normal
human volunteers. The lymphocytes were purified and
red blood cells were removed by Lymphocyte Separation
Media (ICN Pharmaceuticals, Inc., Costa Mesa, CA). The
5    cells were then activated overnight in media containing
10 ng/ml PMA, 500 ng/ml ionomycin, and plate-bound
activating antibodies to CD3. Total RNA was prepared
from the activated cells by the Trizol method
(Gibco/BRL) and poly A RNA was isolated by Dynal bead
10   purification. cDNA was made from the isolated poly A
RNA and size selected for largest cDNA fragments. The
size selected cDNA was then ligated into the plasmid
pSPORT (Gibco/BRL). DNA encoding human CRP1 protein is
obtained by screening the activated lymphocyte cDNA
15   library by either recombinant bacteriophage plaque, or
transformed bacteria colony hybridization protocols
(Sambrook et al. *Supra*). The phage or plasmid cDNA
library are screened using radioactively-labeled probes
derived from the murine CRP1 gene clone as described in
20   Example 1 and Figure 1. The probes are used to screen
nylon filters lifted from the plated library. These
filters are prehybridized for 4 hr at 42°C in 50%
formamide, 5X SSPE, 2X Denhardt's solution, 0.5% SDS,
and 100 µg/ml salmon sperm DNA and then hybridized for
25   24 hr at 42°C in 50% formamide, 5X SSPE, 2X Denhardt's
solution, 0.5% SDS, 100 µg/ml salmon sperm DNA, and 5
ng/ml mB7RP1 probe. The blots are washed in 2X SSC,
0.1% SDS for 10 min at RT, 1X SSC, 0.1% SDS for 10 min
at 50°C, 0.2X SSC, 0.1% SDS for 10 min at 50°C, then
30   0.2X SSC for 10 min at 50°C again. Inserts obtained
from any human CRP1 clones are sequenced and analyzed
as described in Example 1.


Example 3
35       B7RP1 DNA and Amino Acid Sequence

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A cDNA clone, designated smil1-00003-g5,
contained nucleotide sequence homology to B7.1 (CD80)
and B7.2 (CD86).  Translation of the sequence (Figure

5    2A) and subsequent comparison to known proteins in a
public database revealed 20% amino acid identity with
murine B7.1 (Figure 2B).  This low homology was
significant because murine B7.1 shares only 24% amino
acid identity with murine B7.2.  Despite this low

10   homology, critical cysteine residues are conserved
between the open reading frame of this clone and murine
B7.1 and B7.2 at residues 62, 138, 185, and 242
(relative to the initiating methionine, Figure 2B).
The approximate mature protein length and the location

15   of the transmembrane region relative to the carboxy
terminus are also similar in the putative ORF of this
clone, as compared to B7.1 and B7.2.  We named the gene
B7RP1, for B7-Related Protein-1.


20                          Example 4
                   Cloning of Human B7RP1 cDNA


A Genbank blast homology search (GCG,
University of Wisconsin) using murine B7RP1 sequence

25   (see Figure 2) retrieved a clone (AB014553) containing
a 4358 bp sequence with 1679 bp of ORF.  PCR cloning
primers were designed according to this sequence.  A
DNA fragment of 1313 bp was obtained by 5' and 3' RACE
using Human Lymph Node Marathon-Ready™ cDNA (Clontech,

30   Palo Alto, CA) according to the manufacturer's
recommended procedures.
Primers used for full length human B7RP1:

2083-75    ACC ATG CGG CTG GGC AGT CCT GGA
35         (SEQ ID NO: 25)
2083-76    TGG TGA CCT ACC ACA TCC CAC AG

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```
            (SEQ ID NO: 26)
2083-77    TCC GAT GTC ATT TCC TGT CTG GC
            (SEQ ID NO: 27)
2083-78    GCT CTG TCT CCG GAC TCA CAG CCC
 5          (SEQ ID NO: 28)
2113-29    GTG GCA GCA AAC TTC AGC GTG CCC GTC G
            (SEQ ID NO: 29)
2113-30    CCC AAC GTG TAC TGG ATC AAT AAG ACG G
            (SEQ ID NO: 30)
10  2113-31    GCG TGC TGA GGA TCG CAC GGA CCC CCA G
            (SEQ ID NO: 31)
```

Primers 2083-75 and 2083-76 were used to amplify the 5'
end of the gene using RACE protocols.  Primers 2083-77,
15  2083-78, 2113-29, 2113-30, and 2113-31, were used to
amplify the 3' end of the gene using RACE protocols.
        The resulting nucleotide sequence contained
an ORF of 288 amino acid residues beginning at the
methionine.  The predicted mature human B7RP1 amino
20  acid sequence was then compared to the mature mouse
B7RP1 amino acid sequence (Figure 3B) and found to
share 48% amino acid identity.  This homology is
significant because the homology between species is low
with the CD80 (B7.1) gene, in fact, the mouse and human
25  CD80 share only 41% amino acid identity.  Importantly,
the human B7RP1 protein conserve critical cysteine
residues necessary for Ig loop structures (amino acid
residues 16, 92, 138, 194, and 195, relative to the
mature protein, Figure 3B).  In addition, the overall
30  length and position of the transmembrane domain are
consistent with a human B7RP1 homolog.

Example 5
Expression of B7RP1 RNA

35

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RNA in situ hybridization using RNA probes to
the B7RP1 gene.  Adult mouse tissues were fixed in 4%
paraformaldehyde, embedded in paraffin, and sectioned
at 5 μm.  Prior to in situ hybridization, tissues were
5  permeabilized with 0.2M HCL, followed by digestion with
Proteinase K, and acetylation with triethanolamine and
acetic anhydride.  Sections were hybridized overnight
at 55°C with a 969 base $^{33}$P-labeled riboprobe
corresponding to nucleotides 1 to 969 of the mouse
10  B7RP1 sequence.  Excess probe was removed by RNase
digestion, followed by a series of washes in buffer
with decreasing salt concentrations, and then a high
stringency wash in 0.1X SSC at 55°C.  Slides were
dipped in Kodak NTB2 emulsion, exposed at 4°C for 2-3
15  weeks, developed, and counterstained with hematoxilyn
and eosin.  Sections were examined with darkfield and
transmitted light illumination to allow simultaneous
evaluation of the tissue morphology and the
hybridization signal.
20      The analysis of the B7RP1 RNA by in situ
hybridization showed that the B7RP1 RNA was highly
expressed in areas of lymphoid maturation and
lymphocyte activation.  B7RP1 RNA was expressed in the
lymphoid tissues of the thymus, Peyer's patches of the
25  intestine, spleen, and lymph nodes.  Expression within
these lymphoid tissues demonstrated that the B7RP1 RNA
was generally expressed in the areas of B-cell and
other APC involvement.  These regions include the
medulla area of the thymus, the primary follicles of
30  the lymph nodes, and the follicular and dome regions of
the Peyer's patches.  The expression of B7RP1 RNA is
highly specific to the regions of APC involvement in
lymphoid tissues.
        The analysis of several non-lymphoid tissues
35  also revealed B7RP1 expression in regions of APC

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involvement.  In the lung, B7RP1 expression was found
in the submucosal regions, consistent with a function
in antigen processing.  In the small intestine, B7RP1
RNA was found in the lamina propria.  Notably, we found

5  a section of damaged liver, which showed lymphocyte
infiltration that overlapped with the expression of
B7RP1 RNA.  This coincidence of B7RP1 expression with
lymphocyte accumulation in response to tissue damage
strongly indicates that B7RP1 is involved in lymphocyte

10  activation.


Example 6
Expression of CRP1 RNA


15          RNA in situ hybridization using RNA probes to
the CRP1 gene.  Mouse tissues were prepared as in
Example 5. Tissue permeabilization, probe
hybridization, slide treatment, and tissue staining
were as described in Example 5.  Sections were

20  hybridized overnight at 55°C with a 603 base $^{33}$p-
labeled riboprobe corresponding to nucleotides 1 to 603
of the mouse CRP1 sequence.  Sections were examined
with darkfield and transmitted light illumination to
allow simultaneous evaluation of the tissue morphology

25  and the hybridization signal.
          Lymph nodes from normal mice or a mouse
treated with oxazolone were sectioned and analyzed for
CRP1 RNA expression.  The sensitized mouse lymph node
showed greater expression of CRP1 RNA than the normal

30  mouse lymph node.  The expression of CRP1 was in the
paracortex, a region of T-cell activity.  Therefore,
the expression of CRP1 RNA is consistent with that of
T-lymphocyte expression and is up-regulated upon T-cell
activation.


35

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## Example 7
### Expression and Purification of
### CRP1-Fc and B7RP1-Fc Fusion Proteins

5

        To construct the DNA expression vector for
the CRP1- Fc fusion protein, the coding sequence for
the first amino terminal 147 amino acids of the CRP1
was fused, inframe, to the coding sequence for the
10    carboxy terminal 235 amino acids of the human Fc gene
(isotype IgG1) and ligated within the polylinker
sequence of pcDNA3 (pcDNA3/CRP1-Fc). To construct the
DNA expression vector for the B7RP1- Fc fusion protein,
the coding sequence for the first amino terminal 269
15    amino acids of the B7RP1 was fused, inframe, to the
coding sequence for the carboxy terminal 235 amino
acids of the human Fc gene (isotype IgG1) and ligated
within the polylinker sequence of pcDNA3 (pcDNA3/B7RP1-
Fc). The coding sequences of both CRP1 and B7RP1
20    contained sequences from the N-terminus of each protein
up to, but not including, the putative transmembrane
region of each protein. 293T cells were transfected
with either pcDNA3/CRP1-Fc or pcDNA3/B7RP1-Fc using the
FuGene 6 transfection reagent (Roche Molecular
25    Biochemicals, Indianapolis, IN). After four days, the
conditioned media were collected and the Fc fusion
proteins were purified by batch chromatography using
Protein A Sepharose (Pharmacia). Fc fusion proteins
bound to the column were eluted with three column
30    volumes of Immunopure Gentle Elution Buffer (Pierce),
and then were dialyzed against 150 volumes of 20 mM
HEPES, 100 mM NaCl, pH 7.5. The dialyzed protein was
concentrated using Macrosep centrifugal concentrates,
30 kD MWCO (Pall Filtron), and the protein
35    concentrations were calculated using extinction
coefficients derived from the amino acid sequence of

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each protein. Expression of CRP1-Fc fusion protein is shown in Figure 4A, expression of B7CPR1-Fc fusion protein is shown in Figure 4B.


5                           Example 8
                    Identification of CRP1 and B7RP1
                       as a Receptor-ligand Pair


            In order to determine whether the novel
10   proteins were part of the same costimulatory pathway as
     that containing CD28, CTLA-4, B7.1, and B7.2, we
     utilized a cell surface display assay. This assay uses
     ACAS (Adherent Cell Analysis and Sorting) analysis to
     analyze whether membrane-bound proteins expressed in
15   cells interact with various Fc fusion proteins. Cells
     expressing membrane-bound proteins, indicated on the
     left side of Figure 5, were incubated with Fc fusions
     proteins, indicated at the top of the figure.
            Cos-7 cells, grown in DMEM media with 10%
20   FBS, were plated at 500,000 cells/well in a 24-well
     plate. Cells were transfected using the FuGene 6
     reagent (Roche Molecular Biochemicals, Indianapolis,
     IN). For each transfection, 3 µl of FuGene 6 reagent
     was added to 47 µl of serum free DMEM media. After a
25   10 min incubation at room temperature, the mix was
     added to 0.25 µg of plasmid dropwise and then was
     incubated for 15 minutes. The above mix was then added
     to the cells with 0.5 ml of DMEM with 10% FBS. The
     cells were incubated at 37°C in a 5% CO2 atmosphere.
30   As a control, CHO D- cells, stably transfected with an
     expression plasmid containing the cDNA for human CD28,
     were also plated at 500,000 cells/well in a 24-well
     plate.
            After 48 hr, the medium with transfection
35   reagent was removed and the cells were washed twice

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with RPMI plus 5% FBS.   10 to 20 ng of purified Fc
fusion proteins in 1 ml of media were added to the
cells, which were incubated for 30 min on ice.   The
cells were washed three times with RPMI plus 5% FBS and
5   then were incubated with 2 µl of FITC-conjugated anti-
human Fc antibody (1 mg/ml) for another 30 min on ice.
After three successive washes with RPMI, the cells were
covered with 250 µl of RPMI media without phenol red
for ACAS analysis.
10       ACAS analysis of the cells that bound the
various Fc fusion proteins demonstrated that the B7RP1
protein bound CRP1, but not the proteins in the known
costimulatory pathway, CD28 or CTLA-4.   Conversely,
CRP1 interacted with B7RP1, but not B7.2, a component
15   in the known pathway. (See Figure 5).   These results
strongly indicate that CRP1 and B7RP1 represent a novel
receptor-ligand pair, analogous to CD28 and B7.2.
However, since CRP1 and B7RP1 do not interact with
B7.2, CTLA-4, or CD28, they are separate and
20   independent of the known costimulatory pathway.


Example 9
Identification of Cells Expressing B7RP1 Receptors
25
The B7RP1-Fc fusion protein was utilized to
detect cells that expressed receptors to B7RP1,
presumably including the CRP1 protein (see Example 6),
by FACS analysis.   Spleens were removed from female
30   C57/Black 6 mice, ground on 100 micron mesh filters to
release the lymphocytes, passed through 70 micron
filters, and then washed in 50 ml of RPMI-1640.   They
were pelleted at 1500 rpm, resuspended in fresh RPMI,
mixed to break up the clumping cells, and passed
35   through a 40 micron filter.   T-cells to be activated
were seeded into 6 well plates in RPMI-1640, 5% FBS,

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1XPSG, PMA, ionomycin, and incubated at 37°C, 5% CO2
overnight.  T-cell activation was checked by visual
confirmation after 12 hr.

5    Activated spleen cells for immunostaining
were washed in PBS, 0.5% BSA (Path-ocyte 4, ICN
Pharmaceuticals) wash buffer, resuspended, and then
aliquoted in 100 µl volumes. 15 µg/ml of either the
CRP1-Fc fusion protein or the B7RP1-Fc fusion protein
was added (1.5 µg/sample) as appropriate, and then the
10   mixtures were incubated on ice for 30 min with
occasional mixing.  The cells were washed twice in 5.0
ml of wash buffer.  Binding of the fusion proteins was
visualized with 2 µg of goat-anti-human (GaHuFc-FITC)
conjugated secondary antibody in a 100 µl volume for
15   cell staining.  Cell marker antibodies conjugated with
PE were added with the GaHUFc-FITC, as well as control
isotype-PE conjugated antibody controls where indicated
(rat isotype).  The samples were incubated on ice and
washed as before. Visualization was done by FACScan
20   analysis with gating on the lymphocyte populations.
Double staining with CD4+ antibodies and the B7RP1-Fc
fusion protein indicated that the cells expressed both
the CD4 marker and the receptor to B7RP1, presumably
CRP1 (Figure 6).  Similarly, double staining with CD8+
25   antibodies and the B7RP1-Fc fusion protein demonstrated
that cells expressed both CD8 and B7RP1 receptors
(Figure 6).  We could not reliably detect such double
staining cells in inactivated splenocyte preparations.
Since CD4 and CD8 are T-lymphocyte markers, we can
30   postulate that CRP1 is expressed on activated CD4+ and
CD8+ T-cells.  These data are consistent with the
increased expression of CRP1 RNA in the T-cell regions
of lymph nodes from sensitized mice as compared to
normal mice (Example 6).

35

Example 10

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Identification of Cells Expressing CRP1 Ligands

The CRP1-Fc fusion protein was utilized to
detect cells that expressed ligands to CRP1, presumably
5   including the B7RP1 protein (see Example 8), by FACS
analysis (Figure 7). Splenocytes were prepared as in
Example 8, except the 12 hr T-cell activation step was
omitted and the cells were directly analyzed.
Splenocytes were double stained with CD45R (B220)
10  marker antibodies and the CRP1-Fc fusion protein.
Cells were detected that expressed both the CD45R
B-cell marker and the putative ligands for CRP1,
presumably including B7RP1 (Example 8). Therefore, we
conclude that B7RP1 is expressed on B-cells, a type of
15  antigen-presenting cell. These data are consistent
with the expression of B7RP1 RNA in B-cell regions of
various lymphoid tissues (Example 5).
FACS analysis of the expression of B7RP1 on
peritoneal macrophages (Figure 8). Peritoneal cells
20  were collected by local lavage from a normal mouse and
washed before being incubated with the CRP1-Fc fusion
protein or the Fc protein as a control or with the
F4/80 monoclonal antibody (which detects an antigen
specific for macrophages) or an irrelevant, isotype-
25  matched control monoclonal antibody. Cells were then
washed again and incubated with goat-anti-human Fc-FITC
conjugated antibody. After further washing, cells were
assessed in a FACS analyzer for their light scattering
and fluorescence staining properties. Peritoneal cells
30  were first distinguished in subsets on the ground of
their light scattering properties (Figure 8A).
Macrophages were identified in region 5 (R5) because of
their ability to strongly scatter light forward (FSC)
and sideways (SSC) and because of their positive
35  staining for the F4/80 antigen, a marker for
macrophages (Figure 8B). Macrophages in region 6 (R6)

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were singled out on the basis of their less intense
staining for the F4/80 antigen and found to be stained
by the CRP1-Fc fusion protein (Figure 8C). These data
indicate that ligands for CRP1, possibly including
5    B7RP1, are expressed on macrophages, a professional
antigen presenting cell. This is consistent with CRP1
and B7RP1 function in T-lymphocyte activation.


                          Example 11
10        In vitro Inhibitory Activity of the B7RP1-Fc Fusion
                Protein on ConA-Stimulated T-Lymphocytes


             Mouse splenocytes were prepared as in Example
    8 and enriched for T-lymphocytes by negative selection
15   (R and D Systems, Inc., Minneapolis, MN)). 200,000
    splenocytes were used in T-cell proliferation assays in
    a 96-well round-bottom plate. Cells were incubated for
    1 hr with media (no adds), CRP1-Fc, B7RP1-Fc, or B7.2-
    Fc, fusion proteins as indicated Figure 9. Media (no
20   adds), or Con A at various concentrations were added as
    indicated in at the bottom of Figure 9. The cells were
    then incubated at 37°C and 5% $CO_2$. After 42 hr, cells
    were pulsed with 3H-thymidine for 6 hr, harvested and
    incorporated radioactivity determined. Average CPM and
25   standard deviation from triplicate samples are
    represented in Figure 9.
             The Fc fusion proteins did not demonstrate
    significant T-cell stimulatory or inhibitory activity
    by themselves, however, in the presence of 1 µg/ml and
30   3 µg/ml Con A, both the B7RP1-Fc and the known B7.2-Fc
    fusion proteins showed significant inhibitory activity
    (Figure 9). At high concentrations (10 µg/ml), Con A
    stimulation results in cell death, presumably through
    over-activation of the T-cells. Addition of either
35   B7RP1-Fc or B7.2-Fc, significantly protected the cells
    from the detrimental effects of high concentrations of

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Con A.   In both inhibitory and protective functions,
the effect by B7RP1-Fc protein was greater than B7.2-Fc
protein on the Con A stimulated cells.   These data
indicate that the B7RP1 protein functions to regulate
5  T-cell proliferation.


Example 12
Systemic delivery of B7RP1-Fc fusion protein in
transgenic mice

10

The B7RP1-Fc fusion protein described in
Example 7 was subcloned into an ApoE-liver specific
expression vector (Simonet et al. J. Clin. Invest. 94,
1310-1319 (1994) and PCT Application No. US94/11675).
15  The coding region was excised from pCEP4/B7RP1-Fc using
the restriction enzymes, Spe I and Not I, and the
fragment subcloned into the same sites in the
previously mentioned ApoE-liver specific expression
vector.   The resultant plasmid, HE-B7RP1-Fc, was
20  sequenced through it's protein coding region, and
sequences flanking the coding region, to ensure it was
mutation free.
The plasmid was amplified and purified
through two rounds of CsCl density gradient
25  centrifugation.   The purified plasmid DNA was digested
with the restriction enzymes, Cla I and Ase I, and the
1.5 kb transgene insert was purified by agarose gel
electrophoresis.   The purified fragment was diluted to
a stock injection solution of 1 µg/ml in 5 mM Tris, pH
30  7.4, and 0.2 mM EDTA.   Single-cell embryos from BDF1 X
BDF1-bred mice were injected essentially as described
(Brinster et al., Proc. Natl. Acad. Sci. USA 82, 4338
(1985)), except that injection needles were beveled and
siliconized before use.   Embryos were cultured
35  overnight in a CO2 incubator and 15 to 20 2-cell

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embryos were transferred to the oviducts of
pseudopregnant CD1 female mice.

Following term pregnancy, 56 offspring were
obtained from implantation on the microinjected
embryos.  The offspring were screened by PCR
amplification of the integrated transgene in genomic
DNA samples.  The target region for amplification was a
369 bp region of the human Apo E intron which was
included in the expression vector.  The oligos used for
PCR amplification were:


5'-GCC TCT AGA AAG AGC TGG GAC-3' (SEQ ID NO: 32)
5'-CGC CGT GTT CCA TTT ATG AGC-3' (SEQ ID NO: 33)


The conditions for the PCR were: 94°C for 2
min, 1 cycle; 94°C for 1 min, 63°C for 20 sec, and 72°C
for 30 sec, 30 cycles. Of the 56 original offspring, 7
were identified as PCR positive transgenic founder
mice.

At 12 weeks of age, nine transgenic founders
(mouse #1, 2, 4, 6, 8, 30, 32, 33, 40) and five
controls (mouse #5, 9, 10, 25, 28) were sacrificed for
necropsy and pathological analysis.  Total cellular RNA
was isolated from the livers of the founder animals and
negative control littermates as described (McDonald et
al. Meth. Enzymol. 152, 219 (1987)).  Northern blot
analysis was performed on these samples to assess the
level of transgene expression.  Approximately 10 μg of
total RNA from each animal was resolved by agarose
electrophoresis denaturing gels (Ogden et al. Meth.
Enzymol. 152, 61 (1987)), then transferred to HYBOND-N
nylon membrane (Amersham), and probed with $^{32}$P dCTP-
labeled mB7RP1-Fc insert DNA.  Hybridization was
performed for 1 hr at 63°C in ExpressHyb Solution
(Clonetech) and 2-4 X 10$^6$ CPM of labeled probe/ml

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hybridization buffer. Following hybridization, blots were washed twice in 2X SSC, 0.1% SDS at room temperature for 5 min each, and then twice in 0.1X SSC, 0.1% SDS at 55°C for 15-20 min each. Expression of the

5   transgene in founder and control littermates was determined following autoradiography.

Northern blot analyses indicated that seven of the transgenic founders expressed detectable levels of the transgene RNA (mouse #1, 2, 6, 8, 32, 33, and

10  40). The negative control mice and three founders (#4, 30, and 31) did not express detectable levels of RNA. Since the B7RP1-Fc fusion protein was determined to be secreted from mammalian cells in culture (Figure 4B and Example 7), expression of the transgene mRNA should be

15  indicative of the level of systemically delivered gene product.

Example 13

Biological activity of B7RP1-Fc fusion protein

20

Seven of the transgenic mice (mouse #1, 2, 6, 8, 32, 33, and 40) and five control littermates (#5, 9, 10, 25, and 28) were sacrificed for necropsy and pathological analysis using the following procedures:

25  Prior to euthanasia, all animals had their identification numbers verified, then were weighed, anesthetized and blood drawn. The blood was saved as both serum and whole blood for a complete serum chemistry and hematology panel. Radiography was

30  performed just after terminal anesthesia by lethal $CO_2$ inhalation, and prior to gross dissection. Tissues were then removed and fixed in 10% buffered Zn-formalin for histological examination. The tissues collected included the liver, spleen, pancreas, stomach,

35  duodenum, ileum, Peyer's patches, colon, kidney, reproductive organs, skin, mammary glands, bone, brain,

heart, lung, thymus, trachea, eosphagus, thyroid/parathyroid glands, jejunum, cecum, rectum, adrenal glands, white and brown fat, sciatic nerve, bone marrow, urinary bladder, and skeletal muscle.

5 Prior to fixation, the whole organ weights were determined for the liver, heart, stomach, kidney, adrenals, spleen, and thymus. After fixation, the tissues were processed into paraffin blocks, and 3 μm sections were obtained.

10      Immunohistochemistry for the B-lymphocyte marker, B220, and the T-lymphocyte marker, CD3, was performed. To detect B220 or CD3 expression, formalin fixed, paraffin embedded, 4 μm sections were deparaffinized and hydrated to deionized water. The

15 sections were quenched with 3% hydrogen peroxide, blocked with Protein Block (Lipshaw, Pittsburgh, PA), and incubated in rat monoclonal antibody to B220 (Pharmingen, San Diego, CA) or rabbit polyclonal antibody to CD3 (Dako, Carpinteria, CA). The

20 antibodies were detected by biotinylated rabbit anti-rat or goat anti-rabbit immunoglobulins, peroxidase conjugated streptavidin, (BioGenex, San Ramon, CA) with DAB as chromagen (Biotek, Santa Barbara, CA). Sections were counterstained with hemaoxylin.

25      In this study, normal clinical signs were reported during the in-life phase of the study. The whole body radiographs of the transgenic mice were comparable to those of the control mice. The overall hematologic parameters of the transgenic mice were

30 comparable to those of the negative control group, although sporadic changes in individual mice were present: transgenic #8 and #40 had increased serum globulin levels (hyperglobulinemia) and #32 and #33 had globulin levels in the high normal range accompanied by

35 albumin levels in the low normal range, which is a pattern commonly seen with chronic antigenic

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stimulation of the immune system.   Organ weights of the
other transgenic mice were not significantly different
from those of the control group.

The following histopathological changes were
5    present in the transgenic mice:   The mesenteric lymph
nodes of the transgenic B7RP1-Fc mice were moderately
to markedly enlarged when compared to the control mice
(Fig. 10A-10D; Fig. 11A-11E).   The cortex had prominent
follicular hyperplasia seen as enlarged secondary
10   follicles (Fig. 10B-11B) with large germinal centers
containing mostly B220+ B cells (Fig. 11D) and a few
scattered CD3+ T cells (Fig. 11F).   The paracortical
(CD3+ T cell) area was also moderately enlarged (Fig.
11B-11F) and the medullary sinuses had slightly
15   increased numbers of fleshy macrophages (sinus
histiocytosis).   The most conspicuous change in the
nodes was present in the medullary cords, which were
mildly to markedly expanded by large numbers of well-
differentiated plasma cells in the B7RP1-Fc transgenic
20   mice (Fig. 10D).   In transgenic mouse #40, small
numbers of scattered Russell bodies (i.e. plasma cells
with prominent, large, round, intracytoplasmic vesicles
containing immunoglobulins) were also found in the
medullary cords (Fig. 10D).   Interestingly, the other
25   internal and peripheral lymph nodes (e.g. cervical,
inguinal) had similar morphologic features of reactive
lymphoid hyperplasia suggestive of a systemic response.
These findings are consistent with a chronic, ongoing
immune stimulation with enhancement of the humoral
30   immune reaction, which leads to B cell proliferation
and terminal differentiation into plasma cells.

The spleen of B7RP1-Fc transgenic mice had
variably enlarged white pulp areas with moderate
reactive lymphoid hyperplasia involving particularly
35   the B-cell secondary follicles with prominent germinal

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centers and periarteriolar T-cell sheaths when compared to the control mice (Fig. 10E-10F). Another conspicuous finding in B7RP1-Fc transgenic mice was minimal to mild plasmacytosis in the marginal zone
5   surrounding the white pulp areas and in the adjacent red pulp. Transgenic mouse #6 had a few scattered Russell bodies (Fig. 10F, inset). The red pulp had mild to moderate extramedullary hematopoiesis, which was comparable to that seen in the control mice (Fig.
10  10E).

      The small intestinal Peyer's patches were mildly to markedly enlarged in the B7RP1-Fc transgenic mice over those of the control mice (Fig. 10G) and had very large follicles with prominent germinal centers,
15  particularly in transgenic mouse #40 and #32 (Fig. 10H). In addition, there was a minimal (in #32) to mild (in #8 and #33) increase in the numbers of lymphocytes and plasma cells (admixed with a mild eosinophil infiltrate in the ileum of mouse #32) in the
20  thickened lamina propria layer of the mucosa, which was present in the small intestine, but more prominent in the colon of the transgenic mice. The large intestinal lymphoid aggregates (GALT) were also slightly more prominent in some B7RP1-Fc transgenic mice
25  (particularly mouse #8 and #2) than in the control group.

      Generally, the other tissues examined, including the thymus, bone marrow, liver, lung, heart, pancreas, kidneys, adrenal gland, thyroid, parathyroid,
30  trachea, reproductive organs, urinary bladder, mammary gland, skin, skeletal muscle, peripheral nerve, brain, esophagus, stomach, small and large intestine, bone (femur/tibia), stifle joint, white and brown fat appeared normal and comparable to the background
35  changes detected in the control mice.

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        The data from this study demonstrate that
overexpression of the B7-related protein Fc chimera
(B7RP1-Fc) in transgenic mice induces a phenotype
characterized by prominent reactive lymphoid
5   hyperplasia detected in the spleen, peripheral and
internal lymph nodes, and gut-associated lymphoid
tissue, as follicular hyperplasia, expansion of T cell
areas and conspicuous plasmacytosis accompanied by
hyperglobulinemia in some animals. The plasmacytosis
10  is accompanied by higher levels of circulating IgG
(mean $\pm$ SD = 597 $\pm$ 298 mg/ml in transgenic mice vs. 209
$\pm$ 80 mg/ml in control littermates, n = 7, P < 0.05, t
test), in particular IgG2a (217 $\pm$ 100 mg/ml vs. 75 $\pm$ 29
mg/ml, n = 7, P < 0.01, t test). The induction of
15  IgG2a is normally associated with a Th1 cytokines such
as IFN-g Thus, B7RP-1 induces B- and T-cell
proliferation and stimulates B-cells to differentiate
into plasma cells and to produce immunoglobulin.
        These changes are consistent with a
20  persistent systemic immune response with
hyperstimulation of the humoral arm of the immune
system which results in B cell stimulation,
proliferation, and differentiation to antibody-
producing plasma cells throughout the lymphoid organs
25  examined.
        We conclude from the marked lymphoid
hyperplasia demonstrated in the B7RP1-Fc transgenic
mice that B7RP1 protein has significant *in vivo*
biological activity, related to immune system
30  stimulation.


                        Example 14
                  Cloning of human B7RP1


35      Normal human circulating peripheral
lymphocytes were separated from red blood cells using

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Lymphocyte Separation Medium (ICN Pharmaceuticals). The
T-cells were then activated with 10 µg/ml plate bound
anti-CD3 antibody (Immunotech, Westbrook, ME), 10 ng/ml
PMA, and 500 ng/ml ionomycin overnight (16 hours) at
5    37°C and 5% $CO_2$.  Total RNA was then prepared from the
cells using TRIzol reagent (Gibco BRL).  The cells were
pelleted by centrifugation and the cell pellet was
resuspended in 1 ml TRIzol reagent for each 5 X $10^6$
cells and incubated at room temperature for 5 min. 0.2
10   ml chloroform per 1 ml original TRIzol reagent was then
added.  The tubes were shaken vigorously by hand for 15
seconds and incubated for 3 minutes at RT and
centrifuged at 13,000rpm for 15 min at 4°C.  Following
centrifugation, the clear upper aqueous phase which
15   contains the RNA was collected and the sample RNA was
precipitated by the addition of isopropyl alcohol.  The
solution was then incubated at RT for 10 min, the RNA
pelleted, washed with 75% ethanol, and then centrifuged
at 15,000 rpm for 5 min at 4°C.  The pellet was air
20   dried, resuspend in RNAse-free water, then aliquoted,
and stored at -80°C until later use.
      The library was constructed using the
SuperScript Plasmid System for cDNA Synthesis and
Plasmid Cloning (Gibco BRL).  Briefly, cDNA inserts
25   with an average size of 2 kb, were ligated into the
pSport vector at Sal1/ Not1 cloning site.  The ligated
plasmids were electroporated into Electromax
transformation competent *E.coli* (Gibco BRL), titered
and plated at fifteen thousand colonies per LB plate
30   (ampicillin 100 µg/ml).  300,000 colonies were lifted
onto colony/plaque screen hybridization transfer
membranes (NEN Life Sciences), denatured in 0.5 N NaOH,
1.5 M NaCl for 5 minutes, then neutralized successively
for 5 minutes each in the following buffers, 1 M Tris
35   HCl pH 8.0, 0.5 M Tris HCl pH 8.0 and 1.5 M NaCl  and
2X SSC.  The filters were then crosslinked by

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ultraviolet irradiation and baked for 30 min at 80°C in
a vacuum oven.  The filters were pre-washed extensively
in 2X SSC at 42°C to remove debris, then prehybridized
at 42°C in 50% formamide, 5X SSPE, 5X Denhardt's

5    solution, 0.5% SDS, 100 μg/ml salmon sperm DNA, for 2
hours.

The human lymphocyte cDNA library was
screened with an 895 bp DNA fragment having nucleotides
1-711 as shown in Figure 3A, 167 bps immediately 5' to

10   the initiator methionize codon in Figure 3A and 17 bps
immediately 3' to position 711 in Figure 3A.  This
upstream 5' sequence of 167 base pairs was obtained by
5' RACE of the HuB7RP1 cDNA (Example 4) and was
released from a TOPO TA vector (Invitrogen, Carlsbad,

15   CA) at the Eco RI restriction enzyme cleavage site.
This insert was twice purified on a 0.8% agarose TAE
gel.  A DNA gel purification kit (Qiagen) was used to
isolate the DNA insert from the agarose.

125 ng of the DNA fragment was labeled with

20   $^{32}$P dCTP (Amersham) following the Redi-Prime 2 (Amersham)
random prime labeling system protocol.  The colony lift
filters were then allowed to hybridize with the probe
at 42°C In the following buffer overnight at 42°C; 50%
formamide, 5X SSPE, 2X Denhardt's solution, 0.5% SDS,

25   100 mg/ml ssDNA.  The specific activity of the probe
was 2.38 X $10^9$ cpm/μg DNA, in approximately 2 ng
labeled probe per ml hybridization buffer.  The probe
was removed and saved for the next round of screening.
The filters were then washed in 2X SSC, 0.1% SDS RT for

30   15 min, followed by 1X SSC, 0.1% SDS at 55°C for 15
min, and 1X SSC, 0.1% SDS at 60°C for 10 min.  The
filters were wrapped in plastic and exposed to
autoradiography film overnight at -80°C with 2
enhancing screens.  Three independent positive clones

35   were identified.  Exposures were aligned to the

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bacterial plates and the positive clones scraped,
diluted and replated on LB plates with ampicillin
100µg/ml, grown overnight as before and the colonies
were lifted, prepared, and probed as described

5      previously.  Three independent clone colonies were
isolated, the DNA was isolated, and DNA sequenced for
each clone in triplicate.

        The full-length of the human B7RP1 protein is
302 amino acids.  The polypeptide length and relative

10     position of the transmembrane domain, is consistent
with other B7 family members.  The human B7RP1 gene has
43% amino acid identity with the mouse clone.  This
degree of homology is significant since the mouse and
human CD80 proteins are only 41% identical.  Notably

15     conserved between the mouse and human genes are the
cysteine residues at amino acid positions 37, 113, 158,
215, and 216.


                            Example 15
20                    Cloning of human CRP1


        A Genbank blast homology search (GCG,
University of Wisconsin) using murine B7RP1 sequence
(see Figure 2) retrieved a genomic clone (Gen Bank

25     Assession NO. AQ022676) containing a 104 bp sequence
that showed high homology with the murine CRP1 gene.
PCR cloning primers were designed to overlap this
sequence.


30     5'-GCA TAT TTA TGA ATC CCA-3'  (SEQ ID NO: 34)
       5'-ACT ATT AGG GTC ATG CAC-3'  (SEQ ID NO: 35)


        Using the above primers, a 151 bp DNA
fragment of the murine CRP1 was PCR amplified using the

35     murine CRP1 plasmid described in Figure 1 and Example 1
as template.  125 ng of the DNA was labeled with 32P

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dCTP (Amersham) following the Redi-Prime 2 (Amersham)
random prime labeling system protocol. The colony lift
filters from human peripheral blood libraries described
in Example 15 were then allowed to hybridize with the
5   probe in the following hybridization buffer overnight
(15 hr) at 41°C, 50% formamide, 5X SSPE, 2X Denhardt's
solution, 0.5% SDS, 100 μg/ml ssDNA. The specific
activity of the probe was 3.52 X $10^9$ cpm/μg DNA, 1.5 ng
labeled probe/ml hybridization buffer. The probe was
10  pulled off and saved for the next round of screening.
The filters were then washed in 2X SSC, 0.1% SDS at RT
for 10 min, followed by 1X SSC, 0.1% SDS at 37°C for 7
minutes, 40°C for 7 minutes, 44°C for 7 minutes, then
50°C for 7 minutes, continually monitoring the rate at
15  which the filters were releasing the labeled probe.
The filters were wrapped in plastic and exposed to film
overnight at -80°C with 2 enhancing screens. This
method revealed 9 possible independent positive clones.
Exposures were aligned to the bacterial plates and the
20  positive clones scraped, deposited into 200 μl SOC, 2
serial dilutions of 1:10 were performed and 70 μl from
the second dilution was replated on LB plates
containing ampicillin at 100 μg/ml and grown overnight
as before. The colonies were lifted, prepared and
25  probed as before. Eight independent clones were
isolated and DNA prepared by the Qiagen miniprep
method.

A cDNA clone containing an open reading frame
of 199 amino acids was obtained (Figure 13A). This
30  cDNA clone contained nucleotide and amino acid
homologies to the murine CRP1 clone described in
Example 1 and Figure 1. The nucleotides corresponding
to the open reading frame of this human clone was 77%
identical to the murine CRP1 gene. Translation of the
35  human sequence and subsequent comparison with the

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murine CRP1 protein revealed 69% amino acid identity
with the murine protein (Figure 13B). In addition, the
motif between amino acids 114 to 119, "FDPPPF", was
conserved between the murine and human CRP1 genes.
5    This motif corresponds to the "MYPPPY" motif in murine
and human CD28 that is essential for B7 protein
interaction. Furthermore, the cysteines at amino acid
positions 42, 109, and 141 are also conserved. These
cysteines correspond to cysteines in CD28 and CTLA-4 at
10   are involved in Ig loop formation and intermolecular
disulfide dimerization. The close similarity with
murine CRP1, and structural similarities with the CD28
homology family, indicate that this is the human CRP1
homolog.
15


                        Example 16
        CRP-1 is expressed on resting memory T-lymphocytes


20          In order to study CRP-1 expression on memory
T-cells, splenic T-cells were collected from 6-7 month
old mice. These cells were double-stained using B7RP-
1-Fc labeled by an FITC-conjugated anti-human Fc
antibody and a PE-conjugated antibody to either CD44,
25   CD45RB, or CD69. Staining with the B7RP-1-Fc fusion
protein detects expression of CRP-1 protein on these T-
cells. Older mice show more CRP-1+ splenic T-cells
than younger mice. Interestingly, a conspicuous number
of these cells are CD44 high(Fig 14a) and CD45RB low
30   (Fig 14b), a profile typical of memory T-cells. These
CRP-1+ memory T-cells are in a resting state, since
they do not express the activation marker CD69 (Fig.
14c). The expression of CRP-1 on memory T-cells
indicates that CRP-1 has costimulatory functions on
35   memory T-cells.

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Example 17

*In vitro* T-cell costimulation
inhibited by antibodies to B7RP-1.


5          To determine if the B7RP-1 protein has
functional relevance to T-cells, we incubated CD3+ T-
cells with the B7RP-1-Fc fusion protein and an anti-CD3
antibody in an *in vitro* proliferation assay.  Rabbit
anti-mouse B7RP1 polyclonal antibodies or rat anti-
10  mouse B7RP1 monoclonal antibodies were then used to
specifically inhibit B7RP1-Fc costimulated·
proliferation *in vitro*.


B7RP-1 rabbit polyclonal antiserum preparation

15         Three New Zealand white rabbits (5-8 lbs.
initial weight) were injected IM with murine B7RP1
protein. Each rabbit was immunized on day 1 with 150 μg
of murine B7RP1 protein emulsified in an equal volume
of Hunters Titer Max complete adjuvant.  Further boosts
20  (days 14 and 28) were performed by the same procedure.
Antibody titers were monitored by EIA.  After the
second boost, the antisera revealed moderate antibody
titers.  A 30ml production bleed was then obtained from
each animal.  This was repeated each week for 6 weeks.
25  Polyclonal antibodies were then purified by protein-A
agarose chromatography, followed by negative selection
Fc protein affinity chromatography and positive
selection by B7RP-1-Fc affinity chromatography.


30  Rat anti-murine B7RP1 monoclonal antibody preparation
                Rat anti-murine B7RP1 monoclonal antibodies
were generated as described in Practical Immunology,
second edition (1980; L. Hudson and F.C. Hay; Blackwell
Scientific Publications; St. Louis, MO).  Briefly, Lou
35  rats (Harlan; Indianapolis, IN) were injected

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intraperitoneally with muB7RP1-Fc fusion protein
emulsified in Freund's Adjuvant at 4 week intervals.
Three days prior to fusion, rats were boosted
intravenously with soluble muB7RP1. On the day of
5       fusion, the animal was sacrificed under carbon dioxide
and the spleen removed aseptically. Single cell
suspension was generated using a tissue stomacher.
Both splenocytes and Y3-Ag1.2.3 myeloma cells (American
Type Culture Collection; Rockville, MD) were washed in
10      serum-free media then fused by the addition of
polyethylene glycol (PEG 1500; Boehringer Mannheim
Biochemicals; Indianapolis, IN). The cells were rinsed
once, resuspended in serum-containing media, and plated
into 96-well tissue culture plates. Ten to 12 days
15      later, media from each well was tested for specific
antibody to B7RP1 via a direct Enzyme-linked
Immunosorbent Assay (EIA). Cells from wells indicating
potential binding were grown to 10 ml cultures and
frozen in liquid nitrogen. Media from each culture was
20      further tested in flow cytometry and in a functional T-
cell proliferation assay. Those determined to be of
interest by these methods were plated to single cell
colonies, selected again by EIA, and final cell lines
maintained for antibody generation. Antibodies were
25      purified from the cell media by protein A agarose
chromatography.

T-cell preparation and T-cell proliferation assay
                T-cells from the spleens of C57Bl/6 mice (8-
30      12 week-old females, Charles River Laboratories) were
purified by negative selection through a murine T-cell
enrichment column (R&D Systems). The T-cells were then
either used directly or further purified by antibody
and complement lysis as follows. Cells were
35      resuspended (2.5 X $10^6$ cells/ml) in RPMI medium
containing antibodies (all at 10 µg/ml and from

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Pharmingen) against murine CD11b (Clone M1/70), NK-1.1
(Clone PK136), CD8a (Clone 53-6.7), I-A$^b$ (Clone
M5/114.15.2), CD11c (Clone HL3), and the B220 antigen
(Clone RA3-6B2). The cells were then incubated on ice
5    for 30 min, pelleted at 1200 rpm, resuspended in 4:1
vol/vol of RPMI: rabbit complement (Sigma, #S-7764),
and incubated for an additional 30 min at 37°C. The
cells were pelleted again, and the complement treatment
was repeated. Before plating, the cells were washed
10   with RPMI containing 10% FCS. U-bottomed 96 well
plates were coated with an anti-CD3 antibody (Clone
145-2C11, Pharmingen) at concentrations ranging between
0 and 1.2 µg/ml), and anti-human IgG Fab$_2$ (Sigma, 12.5
µg/ml) overnight at 4°C, followed by a 6-9 hr
15   incubation at 37°C. T-cells (1x 10$^5$/well) were
cultured in the absence or presence of various Fc
fusion proteins for 48 hr and were pulsed during the
last 18 hours with 1 µCi of $^3$H-thymidine. Control Fc
proteins included a fusion protein of OPG and Fc and a
20   nonfused Fc protein fragment. The cells were then
harvested and the incorporated radioactivity was
counted. B7RP-1-Fc co-stimulates T-cells to proliferate
in a dose-dependent fashion (Fig. 15a), and an anti-
B7RP-1-Fc antibody specifically inhibits this co-
25   stimulation dose-dependently (Fig. 15b).


                        Example 18
        Inhibitors of the CRP-1/B7RP-1 pathway decrease the
30       onset of rheumatoid arthritis induced by collagen.


            Collagen-induced arthritis (CIA) is an animal
model of autoimmune polyarthritis in rodents and
primates that has many similarities with rheumatoid
35   arthritis in humans. Immunization with heterologous

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species of type II collagen (CII) induces an autoimmune
response to CII that leads to the development of CIA in
susceptible mouse strains.  Congenic strains of mice
with $H-2^r$ and $H-2^q$ are highly susceptible to CIA.  CIA

5   is mediated by the synergistic effects of both CII-
reactive T cells and antibodies.  Porcine CII (Nabozny
et al., Autoimmunity 20, 51-58 (1995)) was dissolved in
0.01N acetic acid at a concentration of 2mg/ml and then
was emulsified at a 1:1 ratio with CFA (Difco).

10  Arthritis susceptible B10.RIII ($H-2^r$) mice (Jackson
Laboratories, Bar Harbor, ME) were immunized with 100
μl of emulsion intradermally at the base of the tail.
Mice were monitored 2-3 times per week for the
development of arthritis.  Arthritis severity was

15  determined using a grading system for each paw as
follows:0: no arthritis; 1: redness or swelling in 1-3
toes; 2: severe swelling of paw; 3: joint ankylosis.
The score of each limb was summed to give a severity
range from 0 to 12 for each animal.

20           Mice were injected with 100ug (in 200 μL) of
protein intraperitonially twice per week.  The
treatment was begun 1 day after immunization with
porcine CII and was stopped at day 52 post-
immunization.  The experiment was conducted in

25  treatment groups of 10 mice, and animals with scores of
1 or above were scored as positive.  The results are
shown in Figure 16 and Table 1.


                          TABLE 1

30     Effect of CRP-1, B7RP-1, CTLA-4, and B7.2 Fc fusion
                 proteins on the onset of arthritis


| Treatment groups | Mean+/-s.d. day of onset |
|---|---|
| CTLA4-Fc | 60.0±0.0 |

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CRP1-Fc.                          48.9±13.2

B7.2-Fc                           28.4±14.1
B7RP1-Fc                          33.9±16.6

PBS                               37.7±17.1


        In mice treated with CRP1-Fc fusion protein,
the onset of arthritic symptoms were delayed by
approximately 10 days as compared to the PBS treated
mice.  This demonstrates that the inhibition of the
CRP-1/B7RP-1 pathway can alleviate disease symptoms in
this mouse model of rheumatoid arthritis.
        Mice treated with B7RP-1-Fc or B7.2-Fc showed
an earlier onset of disease (Table 1 and Figure 16a)
with an increase in arthritic severity (Figure 16b) as
compared to the PBS-treated controls.  This indicates
that the B7RP-1-Fc fusion protein enhances the T-cell
immune response.  Such activity may be useful in
generating anti-tumor immunity in vivo.
        The opposite effects by CRP-1-Fc and B7RP-1-
Fc in this mouse model of rheumatoid arthritis indicate
that the pathway can be manipulated to either enhance
or inhibit the disease progression.  Targeting the CRP-
1 protein with soluble B7RP-1-Fc enhances the disease,
while the interaction of soluble CRP-1-Fc with B7RP-1
inhibits the disease symptoms.


                    Example 19

      B7RP-1-Fc induces an inflammatory bowel disease
              phenotype in transgenic mice.


        Persistent overexpression of the B7-related
protein (B7RP1-Fc) in 22-to-25-week-old transgenic
mice(Example 12) induces a striking phenotype of

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inflammatory bowel disease (IBD) with marked thickening
and chronic inflammation of the small and large bowels
(enterocolitis) and weight loss in some animals.
Histologically, the most severe inflammatory changes

5    were found in the proximal and distal colon, with
milder changes in the small intestine. The proximal
colon was markedly thickened with fissuring ulceration,
transmural inflammation, and hypertrophy of the colonic
mucosa, while the distal colon had diffuse mucosal

10   hypertrophy (or focal erosion and glandular atrophy)
without ulceration. The proximal small intestine had
mild to marked mucosal hypertrophy with milder
inflammatory changes, while the distal small intestine
(ileum) had mild mucosal hypertrophy in some animals

15   and atrophy in other mice. The intestinal changes were
most severe and consistently found in the female B7RP-1
transgenic mice, but were also observed in several of
the male transgenic mice in this study.

It is interesting to note that the histologic
20   features found in the proximal colon, including the
fissuring ulceration and the transmural chronic
granulomatous inflammation with multinucleated giant
cells, more closely resemble those seen in Crohn's
disease than ulcerative colitis in humans.

25   Morphologically, this colitis also mimics the IBD
described in mice deficient in interleukin-10, which
develop wasting, anemia, and enterocolitis affecting
their entire intestinal tract (Kuhn et al. 1993; Sartor
1995; Leach et al. 1999). As in the IL-10 knockout

30   mice, the initial changes in the B7RP-1-Fc transgenic
mice consist of mild, focal infiltrates of inflammatory
cells in the lamina propria without colonic epithelial
hyperplasia (Example 13). In older mice, the affected
colonic segments become thickened due to glandular

35   hypertrophy/hyperplasia and chronic inflammation.

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The proximal and distal colons of the B7RP-1-Fc mice
had moderate to severe colitis with histologic features
of inflammatory bowel disease (IBD). The affected
segments of the proximal colon (Figure 17B-17D) were
5    diffusely thickened, due to prominent glandular
epithelial hypertrophy and hyperplasia with elongation
and dilatation of the mucosal glands (Fig. 17B), which
had increased numbers of mitotic figures and rare crypt
abscesses, but retained goblet cells with mucin (Fig.
10   17D). The mucosa had diffuse chronic inflammation in
the lamina propria, which in some animals extended
transmurally to involve the underlying layers of the
gut wall, including the submucosa, muscularis, serosa,
and the adjacent mesenteric fat tissue (Fig. 17B-17C).
15   The inflammatory infiltrates consisted of lymphocytes
(predominantly CD3+, CD44+ T cells), plasma cells, and
epithelioid macrophages (Fig. 17F) mixed with some
neutrophils and occasional multinucleated giant cells
(Fig. 17E), characteristic of chronic granulomatous
20   inflammation. Lymphoid aggregates (mostly B220+ cells
mixed with small numbers of CD3+ cells) were also
present in the mucosa and around smaller blood vessels
in the submucosa and deeper layers, including
mesenteric fat (Fig. 17C). The lumen contained
25   mucopurulent or mucous exudate (Fig. 17D). Severe
evidence of colitis, with multifocal fissuring
ulceration of the mucosa and transmural inflammation
(Fig. 17B-17C), was found in these B7RP-1-Fc transgenic
mice.
30          The distal colon of the B7RP-1-Fc transgenic
mice was also diffusely thickened and hyperplastic with
elongation, basophilia, and dilatation of the colonic
glands (Fig. 18B-18G), some of which contained crypt
abscesses (Fig. 18D and 18F) and mucus. The lamina
35   propria had a mild diffuse inflammatory infiltrate of
lymphocytes (predominantly CD3+, CD44+ cells,

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particularly in the superficial mucosa; Fig. 18E), as well as plasma cells and focal aggregates of epithelioid macrophages mixed with some neutrophils. Lymphoid aggregates (of predominantly B220+ cells; Fig.
5    18D and 18F) were also scattered throughout the mucosa. The small intestine of B7RP-1-Fc transgenic mice had more variable changes, including mild to focally marked mucosal and crypt hypertrophy and hyperplasia (Fig. 19B and 19D with crypt/villus ratios ranging from 1:4 to
10   1.5:1, as compared to 1:10 in the control mice) accompanied by a predominantly lymphoplasmacytic infiltrate in the lamina propria. The mucosal hyperplasia was most prominent in the proximal small intestine, including the duodenum (Fig. 19B) and
15   particularly the jejunum (Fig. 19D). The crypt architecture was focally deranged and dysplastic in the most severely affected mice (Fig. 19D). In contrast, the distal small intestines (ileum) of some mice, had mild, patchy villous atrophy of the ileal mucosa (Fig.
20   19F) with blunting, thickening or focal loss of villi (with a crypt:villus ratio of 1:1 or less, instead of the normal ratio of 1:2), while other mice had mild ileal mucosal hypertrophy.

The B7RP-1-Fc fusion protein acts to activate
25   cells that are responsible for eliciting a phenotype very similar to that of human Crohn's disease. This indicates that the cells that may be responsible for the inflammation in Crohn's disease are activated by the B7RP-1-Fc fusion protein. Soluble protein,
30   antibody or small molecule inhibitors of B7RP-1 may therefore be useful in inhibiting IBD.


Example 20
35   The B7RP-1-Fc fusion protein
inhibits tumor growth in mice

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To examine the effect of B7RP-1 and CRP-1
on the growth of the immunogenic murine Meth A
sarcoma, we investigated whether the soluble B7RP-
5    1-Fc affects the growth of an established Meth A
sarcoma in Balb/c mice.

Exponentially growing Meth A sarcoma
cells were implanted by intradermal injection of
0.5 million cells in the abdomen of Balb/c mice on
10   day 0.  On day 7, when the tumors reached ~ 100
mm$^3$, the mice were treated with either vehicle
(PBS) or B7RP-1-Fc (8mg/kg), subcutaneously in the
neck on days 7, 10, 14, and 17.  The bidimensional
diameters of the tumors were measured by calipers
15   and the tumor volume (in mm$^3$) was estimated using
the formula:  Tumor volume = [{(width)2xlength}/2].
The tumor growth was monitored up to day 28.  Each
group had eight mice.

The Meth A sarcoma growth pattern of the
20   control tumor was bi-phasic:  a slow initial phase
was followed by a relatively rapid exponential
phase.  In B7RP-1-Fc treated mice, the growth of
the tumor was significantly slower in the rapid
exponential phase.  On day 28, the average volumes
25   of the control and B7RP1-Fc treated mice were 1410
mm$^3$ and 580 mm$^3$, respectively (Figure 20).
Therefore, B7RP-1-Fc treatment inhibited tumor
growth significantly in this model.  The data
strongly suggest the beneficial therapeutic utility
30   of the soluble B7RP-1-Fc protein, and other
activators of the B7RP-1/CRP-1 pathway, in the
treatment of immunogenic tumors.

Immunologic anti-tumor activity is
closely associated with cytolytic T-lymphocyte
35   (CTL) function.  Consistently, the B7RP-1-Fc
protein is expressed on cytolytic CD8+ T-cells

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(Example 9, Figure 6). These data strongly support
B7RP-1 functions on cytolytic CD8+ T-cells. B7RP-1-
Fc, or other stimulators of the B7RP-1/CRP-1
pathway, may therefore be used to enhance cytolytic
5   T-cell and cellular immune functions for a number
of non-cancer-related indications.


## Example 21

10        Inhibition of human B7RP-1 activity in vitro


To determine if human B7RP-1 has positive
co-stimulatory properties, we tested cells
expressing human B7RP-1 and human B7RP-1-Fc fusion
15  protein in T-cell proliferation assays. The human
B7RP-1-Fc fusion protein was constructed by fusing
gene sequences corresponding to amino acids 1 to 247
to a partial human IgG1 gene sequence (Example 14).
The human CRP-1-Fc fusion protein was constructed by
20  fusing gene sequences corresponding to amino acids 1
to 146 to a partial human IgG1 gene sequence
(Example 2). The methods of construction,
expression and purification of both fusion proteins
were conducted as described in Example 7. B7RP-1-Fc
25  demonstrated co-stimulatory activities that are
dependent on anti-CD3 stimulation (Figure 21a). In
addition, this activity can be specifically
inhibited with soluble CRP-1-Fc protein (Figure
21b). Similar co-stimulatory effects were obtained
30  using CHO cells that express membrane-bound, human
B7RP-1, containing the entire coding sequence
(Figure 21c).
        The production of cytokines by human T-
cells under the above in vitro proliferation
35  conditions was determined. Supernatants from T-
cell cultures stimulated for 48 and 72 hours were

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analyzed for IL-2, IL-10, and IFN-gamma by ELISA according to the manufacturer's specifications (BioSource International). The IFN-gamma and IL-10 levels were significantly increased; however,
5   unlike the case with CD28 co-stimulation, IL-2 was not notably induced (Figure 21d). The increased levels of IFN-gamma, a Th1 cytokine, correlate with the B7RP-1 functions to increase IgG2a, as described in Example 13.
10       *In vitro* T-cell co-stimulation assays were conducted as follows. Highly purified human T-cells (>98% CD3+) were isolated by negative selection of fresh or thawed, adherence depleted PBMC using mAb labeled magnetic beads (Miltenyi
15   Biotec). T-cells (1 x $10^5$ cells/well) were cultured in triplicate wells in 96 well plates in 200 µl/well RPMI + 10% FCS. To evaluate B7RP-1-Fc co-stimulation, various concentrations of anti-CD3 (Pharmingen) and 10 µg/ml anti-human IgG Fc (Sigma)
20   in 100 µl 1X PBS were pre-coated onto U bottom plates by an incubation at 4°C overnight. The unbound anti-CD3 and anti-human IgG Fc were removed, and the cells were cultured in the presence or absence of various concentrations of
25   B7RP-1-Fc, OPG-Fc control or anti-CD28 (Pharmingen). For CRP-1-Fc inhibition of B7RP-1-Fc co-stimulation, T-cells were cultured in 0.33 µg/ml anti-CD3 and 10 µg/ml anti-human IgG Fc pre-coated wells with 0.5 µg/ml B7RP-1-Fc in the presence of
30   serially diluted CRP-1-Fc or OPG-Fc, starting at 10 µg/ml. To evaluate co-stimulation by CHO cells expressing B7RP-1, T-cells were cultured in flat bottom plates with various concentrations of soluble anti-CD3 in the presence or absence of

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various amounts of mitomycin-C treated CHO B7RP-1
cells or CHO vector cells.  To test for T-cell
proliferation, cultures were pulsed with 1 uCi/well
[$^3$H]TdR during the last 18 hrs of a 72 hr culture.
5    T-cell proliferation was determined by [$^3$H]TdR
incorporation.  The results of one representative
experiment from three random donors are expressed
as mean CPM incorporated +/- SD.  For analyses of
cytokine production, cells were cultured for 48 and
10   72 hours and supernatants were collected for ELISA.
          These experiments show that the
extracellular portion of human B7RP-1, as described
in Example 14, when fused to a human Fc fragment,
can co-stimulate T-cells in vitro.  This co-
15   stimulation is inhibited by CRP-1-Fc and thus
demonstrates how a soluble inhibitor of human B7RP-
1 may function.  In vitro assays, such as that
described here using human B7RP-1 and CRP-1, could
be used to screen for antibody, soluble protein,
20   peptibody, or small molecule inhibitors of B7RP-
1/CRP-1 activity.


                    *     *     *

25        While the present invention has been
described in terms of the preferred embodiments, it is
understood that variations and modifications will occur
to those skilled in the art.  Therefore, it is intended
that the appended claims cover all such equivalent
30   variations that come within the scope of the invention
as claimed.

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WHAT IS CLAIMED IS:

1. An isolated nucleic acid molecule comprising a
nucleotide sequence selected from the group consisting
5   of:
        a) the nucleotide sequence as set forth in
Figure 1A (SEQ ID NO: 1);
        b) the nucleotide sequence encoding the
polypeptide from residues 1-200 or from residues 21-200
10   as set forth in Figure 1A (SEQ ID NO: 1);
        c) a nucleotide sequence encoding a
polypeptide that is at least about 70 percent identical
to the polypeptide as set forth in Figure 1A (SEQ ID
NO: 1);
15        d) a naturally occurring allelic variant or
alternate splice variant of any of (a), (b) or (c );
        e) a nucleotide sequence complementary to any
of (a), (b) or (c );
        f) a nucleotide sequence of (b),(c ) or (d)
20   encoding a polypeptide fragment of at least about 25,
50, 75, 100, or greater than 100 amino acid residues;
        g) a nucleotide sequence of (a), (b) or (c )
comprising a fragment of at least about 10, 15, 20, 25,
50, 75, 100, or greater than 100 nucleotides; and
25        h) a nucleotide sequence which hybridizes
under stringent conditions to any of (a)-(g).


2. An isolated nucleic acid molecule comprising a
nucleotide sequence selected from the group consisting
30   of:
        a) the nucleotide sequence as set forth in
Figure 2A (SEQ ID NO: 11) or Figure 3A (SEQ ID NO: 6)
or Figure 12A (SEQ ID NO: 16);
        b) the nucleotide sequence encoding the
35   polypeptide as set forth in Figure 2A (SEQ ID NO: 6)
from residues 1-322 or from residues 47-322, or as set

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forth in Figure 3A (SEQ ID NO: 11) from residues 1-288 or from residues 19-288, 20-288, 21-288, 22-288, 24-288, or 28-288 or as set forth in Figure 12A from residues 1-302, or from residues 19-302, 20-302, 21-302, 22-302, 24-302 or 28-302;

           c) a nucleotide sequence encoding a polypeptide that is at least about 70 percent identical to the polypeptide as set forth in Figure 2A (SEQ ID NO: 6) or Figure 3A (SEQ ID NO: 11) or Figure 12A (SEQ ID NO: 6);

           d) a naturally occurring allelic variant or alternate splice variant of any of (a), (b) or (c );

           e) a nucleotide sequence complementary to any of (a), (b) or (c );

           f) a nucleotide sequence of (b),(c ) or (d) encoding a polypeptide fragment of at least about 25, 50, 75, 100, or greater than 100 amino acid residues;

           g) a nucleotide sequence of (a), (b) or (c ) comprising a fragment of at least about 10, 15, 20, 25, 50, 75, 100, or greater than 100 nucleotides; and

           h) a nucleotide sequence which hybridizes under stringent conditions to any of (a)-(g).

    3. The nucleic acid molecule of Claims 1 or 2 wherein the nucleotide sequence is operably linked to an expression control sequence.

    4. A host cell comprising the nucleic acid molecule of Claim 2.

    5. The host cell of Claim 3 which is a eucaryotic cell.

    6. The host cell of Claim 3 which is a procaryotic cell.

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7. A process for producing a polypeptide comprising growing a culture of the host cell of Claim 3 in suitable culture medium and isolating the polypeptide from the culture.

5

8. A polypeptide produced by the process of Claim 7.

9. A polypeptide encoded by the nucleic acid
10  molecule of Claim 1.

10. A polypeptide encoded by the nucleic acid molecule of Claim 2.

15        11. An isolated polypeptide comprising the amino acid sequence selected from the group consisting of:
        a) the amino acid sequence as set forth in Figure 1A (SEQ ID NO: 2);
        b) the mature amino acid sequence as set
20  forth in Figure 1A (SEQ ID NO: 2) comprising a mature amino terminus at residue 21;
        c) a fragment of the amino acid sequence set forth in Figure 1A (SEQ ID NO: 2) comprising at least about 25, 50, 75, 100, or greater than 100 amino acid
25  residues;
        d) an ortholog of (a), (b) or (c ); and
        e) an allelic variant or alternative splice variant of (a), (b), (c) or (d).

30        12. An isolated polypeptide comprising the amino acid sequence selected from the group consisting of:
        a) the amino acid sequence as set forth in Figure 2A (SEQ ID NO: 7) or Figure 3A (SEQ ID NO: 12) or Figure 12A (SEQ ID NO: 17);
35        b) the mature amino acid sequence as set forth in Figure 2A (SEQ ID NO: 7) comprising a mature

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amino terminus at residues 47, or Figure 3A (SEQ ID NO:
12) comprising a mature amino terminus at any of
residues 19, 20, 21, 22, 24 or 28, or Figure 12A (SEQ
ID NO: 17) comprising a mature amino terminus at any of
5    residues 19,20,21,22,24,or 28;
            c) a fragment of the amino acid sequence set
forth in Figure 2A (SEQ ID NO: 7) or Figure 3A (SEQ ID
NO: 12) or Figure 12A (SEQ ID NO: 17) comprising at
least about 25, 50, 75, 100, or greater than 100 amino
10   acid residues;
            d) an ortholog of (a), (b) or (c·); and
            e) an allelic variant or alternative splice
variant of (a), (b), (c) or (d).


15       13. An antibody or fragment thereof specifically
binding the polypeptide of Claims 9, 10, 11 or 12.


         14. The antibody of Claim 11 which is a monoclonal
antibody.
20

         15. The antibody of Claim 13 which is a human
antibody.


         16. The antibody of Claim 13 which is a humanized
25   or CDR-grafted antibody.


         17. The antibody or fragment of Claim 13 which
binds B7RP1 or to a B7RP1 extracellular domain.


30       18. The antibody or fragment of Claim 13 which
inhibits the binding of B7RP1 to CRP1.


         19. A composition comprising the polypeptide of
Claims 9, 10, 11 or 12 and a pharmaceutically
35   acceptable carrier, adjuvant, solubilizer, stabilizer
or anti-oxidant.

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20. A polypeptide comprising a derivative of the polypeptide of Claims 9, 10, 11 or 12.

5       21. The polypeptide of Claim 20 which is covalently modified with a water-soluble polymer.

22. A fusion polypeptide comprising the polypeptide of Claims 9, 10, 11 or 12 fused to a
10     heterologous amino acid sequence.

23. The fusion polypeptide of Claim 22 wherein the heterologous amino acid sequence is an IgG constant domain or fragment thereof.
15

24. A method for treating, preventing or ameliorating a T cell mediated disorder comprising administering to an animal the polypeptide of Claims 9, 10, 11 or 12.
20

25. A method of diagnosing a T cell mediated disorder or a susceptibility to a T cell mediated disorder in an animal comprising:
        a) determining the presence or amount of
25     expression of the polypeptide of Claims 9, 10, 11 or 12; and
        b) diagnosing a T-cell mediated disorder or a susceptibility to a T-cell mediated disorder based on the presence or amount of expression of the
30     polypeptide.

26. A method of identifying a test molecule which binds to a polypeptide comprising:
        a) contacting the polypeptide of Claims 9,
35     10, 11 or 12 with a test molecule; and

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b) determining the extent of binding of the polypeptide to the test molecule.

27. The method of Claim 26 further comprising determining the activity of the polypeptide when bound to the compound.

28. A method of regulating T cell activation or proliferation in an animal comprising administering to the animal the nucleic acid molecule of Claims 1, 2 or 3.

29. A transgenic non-human mammal comprising the nucleic acid molecule of Claim 3.

30. A method of suppressing an immune response in an animal comprising administering to the animal an antagonist of CRP-1 or B7RP-1.

31. The method of Claim 30 wherein the antagonist is an antibody which binds B7RP-1.

## FIGURE 1A

```
ATG AAG CCG TAC TTC TGC CGT GTC TTT GTC TTC TGC TTC CTA ATC          45
 M   K   P   Y   F   C   R   V   F   V   F   C   F   L   I
             5                  10                      15

AGA CTT TTA ACA GGA GAA ATC AAT GGC TCG GCC GAT CAT AGG ATG          90
 R   L   L   T   G  •E   I   N   G   S   A   D   H   R   M
                 20                  25              30

TTT TCA TTT CAC AAT GGA GGT GTA CAG ATT TCT TGT AAA TAC CCT         135
 F   S   F   H   N   G   G   V   Q   I   S   C   K   Y   P
                 35                  40              45

GAG ACT GTC CAG CAG TTA AAA ATG CGA TTG TTC AGA GAG AGA GAA         180
 E   T   V   Q   Q   L   K   M   R   L   F   R   E   R   E
                 50                  55              60

GTC CTC TGC GAA CTC ACC AAG ACC AAG GGA AGC GGA AAT GCG GTG         225
 V   L   C   E   L   T   K   T   K   G   S   G   N   A   V
                 65                  70              75

TCC ATC AAG AAT CCA ATG CTC TGT CTA TAT CAT CTG TCA AAC AAC         270
 S   I   K   N   P   M   L   C   L   Y   H   L   S   N   N
                 80                  85              90

AGC GTC TCT TTT TTC CTA AAC AAC CCA GAC AGC TCC CAG GGA AGC         315
 S   V   S   F   F   L   N   N   P   D   S   S   Q   G   S
                 95                  100             105

TAT TAC TTC TGC AGC CTG TCC ATT TTT GAC CCA CCT CCT TTT CAA         360
 Y   Y   F   C   S   L   S   I   F   D   P   P   P   F   Q
                 110                 115             120

GAA AGG AAC CTT AGT GGA GGA TAT TTG CAT ATT TAT GAA TCC CAG         405
 E   R   N   L   S   G   G   Y   L   H   I   Y   E   S   Q
                 125                 130             135

CTC TGC TGC CAG CTG AAG CTC TGG CTA CCC GTA GGG TGT GCA GCT         450
 L   C   C   Q   L   K   L   W   L   P   V   G   C   A   A
                 140                 145             150

TTC GTT GTG GTA CTC CTT TTT GGA TGC ATA CTT ATC ATC TGG TTT         495
 F   V   V   V   L   L   F   G   C   I   L   I   I   W   F
                 155                 160             165

TCA AAA AAG AAA TAC GGA TCC AGT GTG CAT GAC CCT AAT AGT GAA         540
 S   K   K   K   Y   G   S   S   V   H   D   P   N   S   E
                 170                 175             180

TAC ATG TTC ATG GCG GCA GTC AAC ACA AAC AAA AAG TCT AGA CTT         585
 Y   M   F   M   A   A   V   N   T   N   K   K   S   R   L
                 185                 190             195

GCA GGT GTG ACC TCA                                                 600
 A   G   V   T   S
                 200
```

## FIGURE 1B

```
mCRP1      MKPYFCRVFV FCFLIRLL-- -----TGEIN GS----ADHR MFSFHNGGVQ        39
mCD28      MT-------- ----LRLLFL ALNFFSVQVT ENKILVKQSP LLVVDSNEVS        38
Consensus  M.........  .....RLL..  .......... ..........  ........V.

mCRP1      ISCKYPETV- -QQLKMRLFR --EREV-LCE LTKTKGSGNA VSIKNPMLCL        ?4
mCD28      LSCRYSYNLL AKEFRASLYK GVNSDVEVCV GNGNFTYQPQ FRSNAEFNCD        88
Consensus  .SC.Y..... .......L..  .....V..C. ..........  ........C.

mCRP1      YHLSNNSVSF FLNNPDSSQG SYYFCSLSIF DPPPFQERNL SGGYL-HIYE        133
mCD28      GDFDNETVTF RLWNLHVNHT DIYFCKIEFM YPPPYLDNER SNGTIIHIKE        138
Consensus  .....N..V.F .L.N......  ..YFC.....  .PPP......  S.G...HI.E

mCRP1      SQLC---CQL KL-W-LPVGC AA-FVVVLLF GCIL-IIWFS KKKY----GS        172
mCD28      KHLCHTQSSP KLFWALVVVA GVLFCYGLLV TVALCVIWTN SRRNRLLQVT        188
Consensus  ..LC...... KL.W.L.V..  ...F...LL. ...L..IW..  ..........

mCRP1      SVH-DPNSEY MFMAAVNTNK KSR-LAGVTS                             200
mCD28      TMNMTPRRPG LTRKPYQPYA PARDFAAYRP                             218
Consensus  .....P.... ..........  ..R..A.... ...
```

# FIGURE 2A

```
ATG CAG CTA AAG TGT CCC TGT TTT GTG TCC TTG GGA ACC AGG CAG    45
 M   Q   L   K   C   P   C   F   V   S   L   G   T   R   Q
                 5                  10                  15

CCT GTT TGG AAG AAG CTC CAT GTT TCT AGC GGG TTC TTT TCT GGT    90
 P   V   W   K   K   L   H   V   S   S   G   F   F   S   G
                20                  25                  30

CTT GGT CTG TTC TTG CTG CTG TTG AGC AGC CTC TGT GCT GCC TCT   135
 L   G   L   F   L   L   L   L   S   S   L   C   A   A   S
                35                  40                  45

GCA GAG ACT GAA GTC GGT GCA ATG GTG GGC AGC AAT GTG GTG CTC   180
 A  *E   T   E   V   G   A   M   V   G   S   N   V   V   L
                50                  55                  60

AGC TGC ATT GAC CCC CAC AGA CGC CAT TTC AAC TTG AGT GGT CTG   225
 S   C   I   D   P   H   R   R   H   F   N   L   S   G   L
                65                  70                  75

TAT GTC TAT TGG CAA ATC GAA AAC CCA GAA GTT TCG GTG ACT TAC   270
 Y   V   Y   W   Q   I   E   N   P   E   V   S   V   T   Y
                80                  85                  90

TAC CTG CCT TAC AAG TCT CCA GGG ATC AAT GTG GAC AGT TCC TAC   315
 Y   L   P   Y   K   S   P   G   I   N   V   D   S   S   Y
                95                  100                 105

AAG AAC AGG GGC CAT CTG TCC CTG GAC TCC ATG AAG CAG GGT AAC   360
 K   N   R   G   H   L   S   L   D   S   M   K   Q   G   N
                110                 115                 120

TTC TCT CTG TAC CTG AAG AAT GTC ACC CCT CAG GAT ACC CAG GAG   405
 F   S   L   Y   L   K   N   V   T   P   Q   D   T   Q   E
                125                 130                 135

TTC ACA TGC CGG GTA TTT ATG AAT ACA GCC ACA GAG TTA GTC AAG   450
 F   T   C   R   V   F   M   N   T   A   T   E   L   V   K
                140                 145                 150

ATC TTG GAA GAG GTG GTC AGG CTG CGT GTG GCA GCA AAC TTC AGT   495
 I   L   E   E   V   V   R   L   R   V   A   A   N   F   S
                155                 160                 165

ACA CCT GTC ATC AGC ACC TCT GAT AGC TCC AAC CCG GGC CAG GAA   540
 T   P   V   I   S   T   S   D   S   S   N   P   G   Q   E
                170                 175                 180

CGT ACC TAC ACC TGC ATG TCC AAG AAT GGC TAC CCA GAG CCC AAC   585
 R   T   Y   T   C   M   S   K   N   G   Y   P   E   P   N
                185                 190                 195

CTG TAT TGG ATC AAC ACA ACG GAC AAT AGC CTA ATA GAC ACG GCT   630
 L   Y   W   I   N   T   T   D   N   S   L   I   D   T   A
                200                 205                 210

CTG CAG AAT AAC ACT GTC TAC TTG AAC AAG TTG GGC CTG TAT GAT   675
 L   Q   N   N   T   V   Y   L   N   K   L   G   L   Y   D
                215                 220                 225

GTA ATC AGC ACA TTA AGG CTC CCT TGG ACA TCT CGT GGG GAT GTT   720
 V   I   S   T   L   R   L   P   W   T   S   R   G   D   V
                230                 235                 240
```

# FIGURE 2A (Con't)

```
CTG TGC TGC GTA GAG AAT GTG GCT CTC CAC CAG AAC ATC ACT AGC        765
 L   C   C   V   E   N   V   A   L   H   Q   N   I   T   S
                245                 250                 255

ATT AGC CAG GCA GAA AGT TTC ACT GGA AAT AAC ACA AAG AAC CCA        810
 I   S   Q   A   E   S   F   T   G   N   N   T   K   N   P
                260                 265                 270

CAG GAA ACC CAC AAT AAT GAG TTA AAA GTC CTT GTC CCC GTC CTT        855
 Q   E   T   H   N   N   E   L   K   V   L   V   P   V   L
                275                 280                 285

GCT GTA CTG GCG GCA GCG GCA TTC GTT TCC TTC ATC ATA TAC AGA        900
 A   V   L   A   A   A   A   F   V   S   F   I   I   Y   R
                290                 295                 300

CGC ACG CGT CCC CAC CGA AGC TAT ACA GGA CCC AAG ACT GTA CAG        945
 R   T   R   P   H   R   S   Y   T   G   P   K   T   V   Q
                305                 310                 315

CTT GAA CTT ACA GAC CAC GCC                                        966
 L   E   L   T   D   H   A
                320     322
```

# FIGURE 2B

```
mB7RP1      MQLKCPCFVS LGTRQPVWKK LHVSSGFFSG LGLFLLLLS- SLCAASAETE      49
mCD80       MA--CNC--Q LMQDTPL--- LKFPCPRLI- L-LFVLLIRL SQVSSDVDEQ      41
Consensus   M...C.C... L....P.... L......... L.LF.LL... S.........

mB7RP1      VGAMVGSNVV LSCIDPHRRH FNLSGLYVYW QIENPEVSVT YYLPYKSPGI      99
mCD80       LSKSVKDKVL LPC-RYNSPH EDESEDRIYW QKHDKVV--- --LSVIAGKL      85
Consensus   ....V...V. L.C.....H ...S....YW Q......V... ..L.......

mB7RP1      NVDSSYKNRG HLSLDSMKQG NFSLYLKNVT PQDTQEFTCR VFMNTATELV      149
mCD80       KVWPEYKNR- --TL--YDNT TYSLIILGLV LSDRGTYSCV VQKKERGTYE      130
Consensus   .V...YKNR. ...L...... ..SL...... ..D.....C. V.........

mB7RP1      KILEEVVRLR VAANFSTPVI STSDSSNPGQ ERTYTCMSKN GYPEPNLYWI      199
mCD80       VKHLALVKLS IKADFSTPNI TESGNPSADT KRI-TCFASG GFPKPRFSWL      179
Consensus   .....V.L. ..A.FSTP.I ..S....... .R..TC.... G.P.P...W.

mB7RP1      -NTTDNSLID TALQNNTVYL NKLGLYDVIS TLRLPWTSRG DVLCCVENVA      248
mCD80       ENGRELPGIN TTISQDPESE LYTISSQLDF NTTRNHTIKC LIKYGDAHVS      229
Consensus   .N.....I. T......... ........... ......T... ........V.

mB7RP1      LHQNITSISQ AESFTGNNTK NPQETHNNEL KVLVPVLAVL A-AAAFVSFI      297
mCD80       EDFTWEKPPE DPPDSKNTLV LFGAGFGAVI TVVVIVVIIK CFCKHRSCFR      279
Consensus   .......... ......N... ......... .V.V.V....I.......F.

mB7RP1      IYRRTR-PHR SYT-GPKTVQ LELTDHA                             322
mCD80       RNEASRETNN SLTFGPEEAL AEQTVFL                             306
Consensus   ....R..:. S.T.GP... .E.T...
```

## FIGURE 3A

```
ATG CGG CTG GGC AGT CCT GGA CTG CTC TTC CTG CTC TTC AGC AGC        45
M   R   L   G   S   P   G   L   L   F   L   L   F   S   S
                5           10                      15

CTT CGA GCT GAT ACT CAG GAG AAG GAA GTC AGA GCG ATG GTA GGC        90
L   R   A   *D  *T  *Q  *E  K   *E  V   R   A   *M  V   G
                20                  25                  30

AGC GAC GTG GAG CTC AGC TGC GCT TGC CCT GAA GGA AGC CGT TTT        135
S   D   V   E   L   S   C   A   C   P   E   G   S   R   F
                35                  40                  45

GAT TTA AAT GAT GTT TAC GTA TAT TGG CAA ACC AGT GAG TCG AAA        180
D   L   N   D   V   Y   V   Y   W   Q   T   S   E   S   K
                50                  55                  60

ACC GTG GTG ACC TAC CAC ATC CCA CAG AAC AGC TCC TTG GAA AAC        225
T   V   V   T   Y   H   I   P   Q   N   S   S   L   E   N
                65                  70                  75

GTG GAC AGC CGC TAC CGG AAC CGA GCC CTG ATG TCA CCG GCC GGC        270
V   D   S   R   Y   R   N   R   A   L   M   S   P   A   G
                80                  85                  90

ATG CTG CGG GGC GAC TTC TCC CTG CGC TTG TTC AAC GTC ACC CCC        315
M   L   R   G   D   F   S   L   R   L   F   N   V   T   P
                95                  100                 105

CAG GAC GAG CAG AAG TTT CAC TGC CTG GTG TTG AGC CAA TCC CTG        360
Q   D   E   Q   K   F   H   C   L   V   L   S   Q   S   L
                110                 115                 120

GGA TTC CAG GAG GTT TTG AGC GTT GAG GTT ACA CTG CAT GTG GCA        405
G   F   Q   E   V   L   S   V   E   V   T   L   H   V   A
                125                 130                 135

GCA AAC TTC AGC GTG CCC GTC GTC AGC GCC CCC CAC AGC CCC TCC        450
A   N   F   S   V   P   V   V   S   A   P   H   S   P   S
                140                 145                 150

CAG GAT GAG CTC ACC TTC ACG TGT ACA TCC ATA AAC GGC TAC CCC        495
Q   D   E   L   T   F   T   C   T   S   I   N   G   Y   P
                155                 160                 165

AGG CCC AAC GTG TAC TGG ATC AAT AAG ACG GAC AAC AGC CTG CTG        540
R   P   N   V   Y   W   I   N   K   T   D   N   S   L   L
                170                 175                 180

GAC CAG GCT CTG CAG AAT GAC ACC GTC TTC TTG AAC ATG CGG GGC        585
D   Q   A   L   Q   N   D   T   V   F   L   N   M   R   G
                185                 190                 195

TTG TAT GAC GTG GTC AGC GTG CTG AGG ATC GCA CGG ACC CCC AGC        630
L   Y   D   V   V   S   V   L   R   I   A   R   T   P   S
                200                 205                 210

GTG AAC ATT GGC TGC TGC ATA GAG AAC GTG CTT CTG CAG CAG AAC        675
V   N   I   G   C   C   I   E   N   V   L   L   Q   Q   N
                215                 220                 225

CTG ACT GTC GGC AGC CAG ACA GGA AAT GAC ATC GGA GAG AGA GAC        720
L   T   V   G   S   Q   T   G   N   D   I   G   E   R   D
                230                 235                 240
```

## FIGURE 3A (Con't)

```
AAG ATC ACA GAG AAT CCA GTC AGT ACC GGC GAG AAA AAC GCG GCC      765
K   I   T   E   N   P   V   S   T   G   E   K   N   A   A
            245                     250                 255

ACG TGG AGC ATC CTG GCT GTC CTG TGC CTG CTT GTG GTC GTG GCG      810
T   W   S   I   L   A   V   L   C   L   L   V   V   V   A
            260                     265                 270

GTG GCC ATA GGC TGG GTG TGC AGG GAC CGA TGC CTC CAA CAC AGC      855
V   A   I   G   W   V   C   R   D   R   C   L   Q   H   S
            275                     280                 285

TAT GCA GGT                                                      864
Y   A   G
        288
```

FIGURE 3B

| hB7RP1 | EKEVRAMVGS | DVELSCACPE | GSRFDLNDVY | VYWQTSESKT | VVTYHIPQNS | 50 |
| mB7RP1 | ETEVGAMVGS | NVVLSCIDPH | RRHFNLSGLY | VYWQIENPEV | SVTYYLPYKS | 50 |
| Consensus | E.EV.AMVGS | .V.LSC..P. | H..F.L...Y | VYWQ...... | .VTY..P..S | |

| hB7RP1 | SLENVDSRYR | NRALMSPAGM | LRGDFSLRLF | NVTPQDEQKF | HCLVLSQ-SL | 99 |
| mB7RP1 | PGINVDSSYK | NRGHLSLDSM | KQGNFSLYLK | NVTPQDTQEF | TCRVFMNTAT | 100 |
| Consensus | ...NVDS.Y. | NR...S...M | ..G.FSL.L. | NVTPQD.Q.F | .C.V...... | |

| hB7RP1 | GFQEVLSVEV | TLHVAANFSV | PVVSAPHSPS | Q-DELTFTCT | SINGYPRPNV | 148 |
| mB7RP1 | ELVKILEEVV | RLRVAANFST | PVISTSDSSN | PGQERTYTCM | SKNGYPEPNL | 150 |
| Consensus | .....L...V | .L.VAANFS. | PV.S...S.. | G...E.T.TC. | S.NGYP.PN. | |

| hB7RP1 | YWINKTDNSL | LDQALQNDTV | FLNMRGLYDV | VSVLRIARTP | SVNIGCCIEN | 198 |
| mB7RP1 | YWINTTDNSL | IDTALQNNTV | YLNKLGLYDV | ISTLRLPWTS | RGDVLCCVEN | 200 |
| Consensus | YWIN.TDNSL | .D.ALQN.TV | .LN..GLYDV | .S.LR...T. | ......CC.EN | |

| hB7RP1 | VLLQQNLTVG | SQTGNDIGER | DKITENPVST | GEKNAATWSI | LAVLCLLVVV | 248 |
| mB7RP1 | VALHQNITSI | SQAESFTGNN | TKNPQETHNN | ELKVLV--PV | LAVLAAAAFV | 248 |
| Consensus | V.L.QN.T.. | SQ..?..G.. | .K....K.. | ..K...T... | LAVL...V | |

| hB7RP1 | AVAIGWVCRD | RCLQHSYAG | | | | 267 |
| mB7RP1 | SFIIYR--RT | R-PHRSYTGP | KTVQLELTDH | A | | 276 |
| Consensus | ...I...R. | R....SY.G. | ....~...... | . | | |

Figure 4A
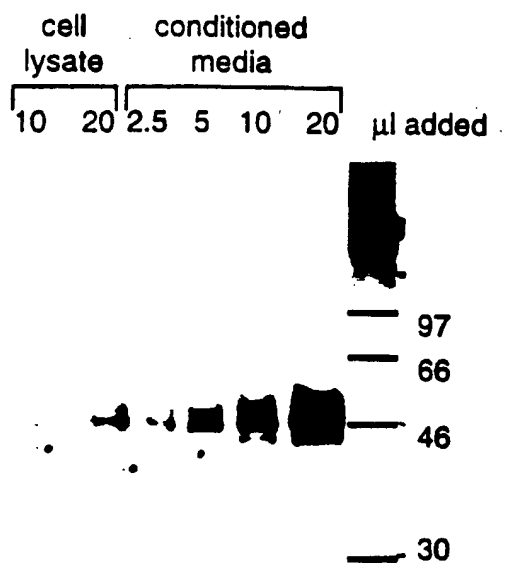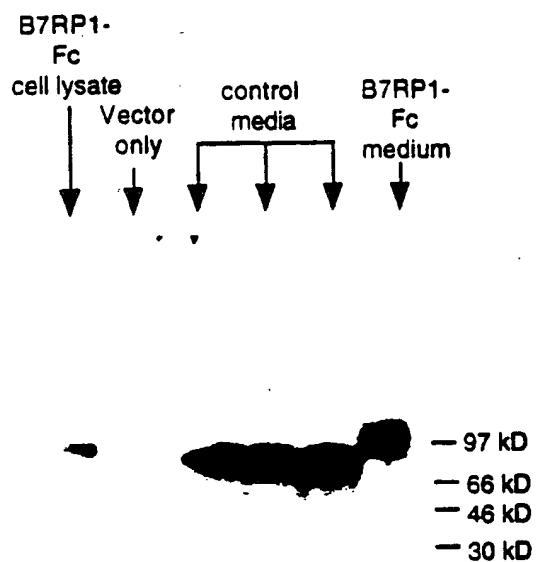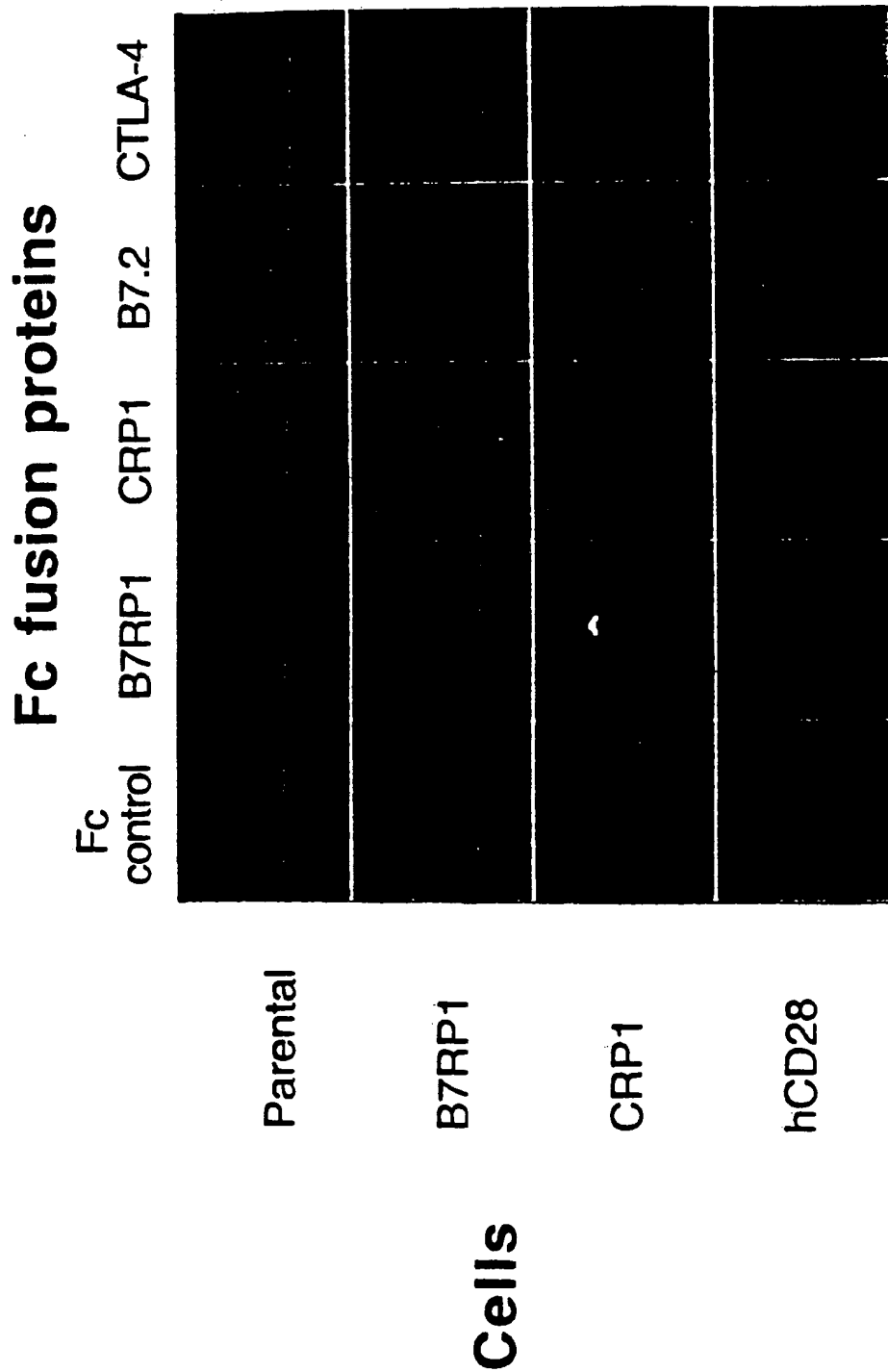
Figure 4B
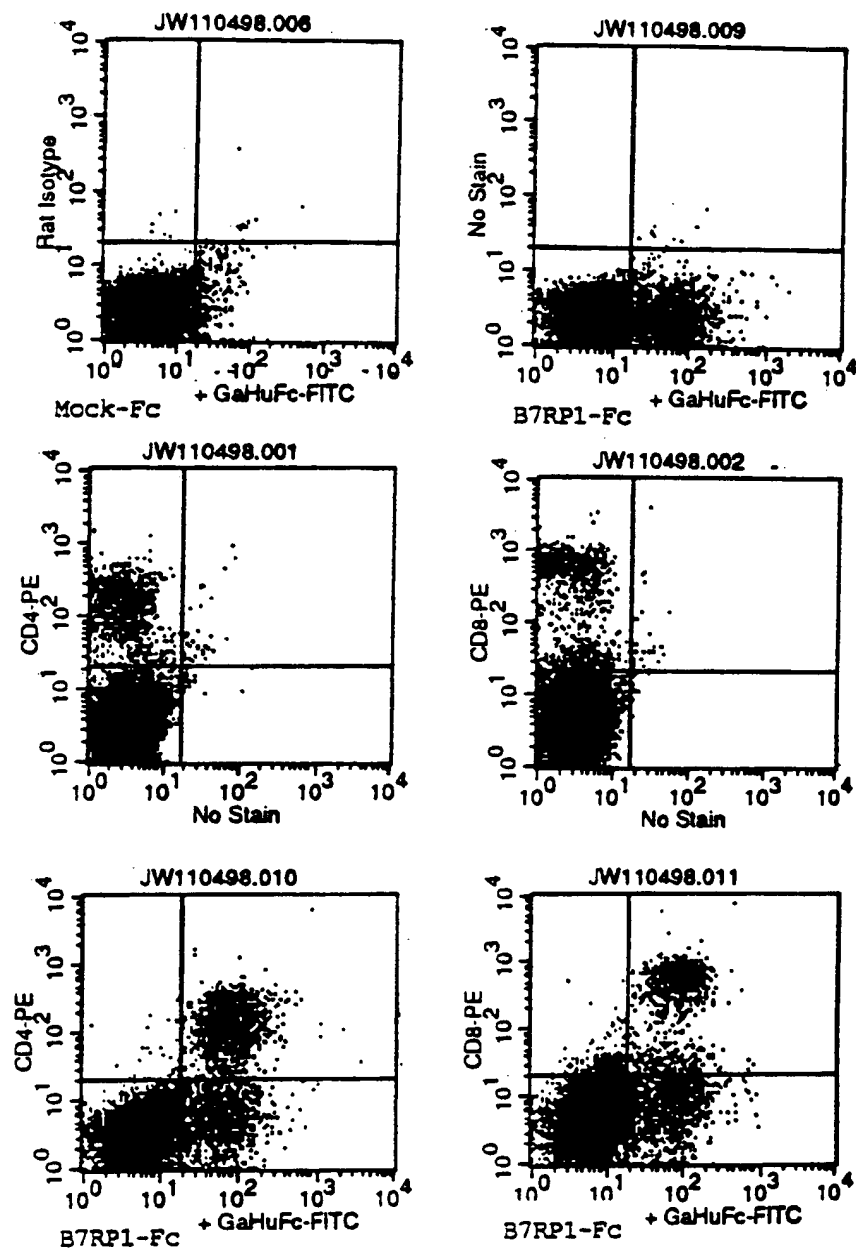
Figure 5
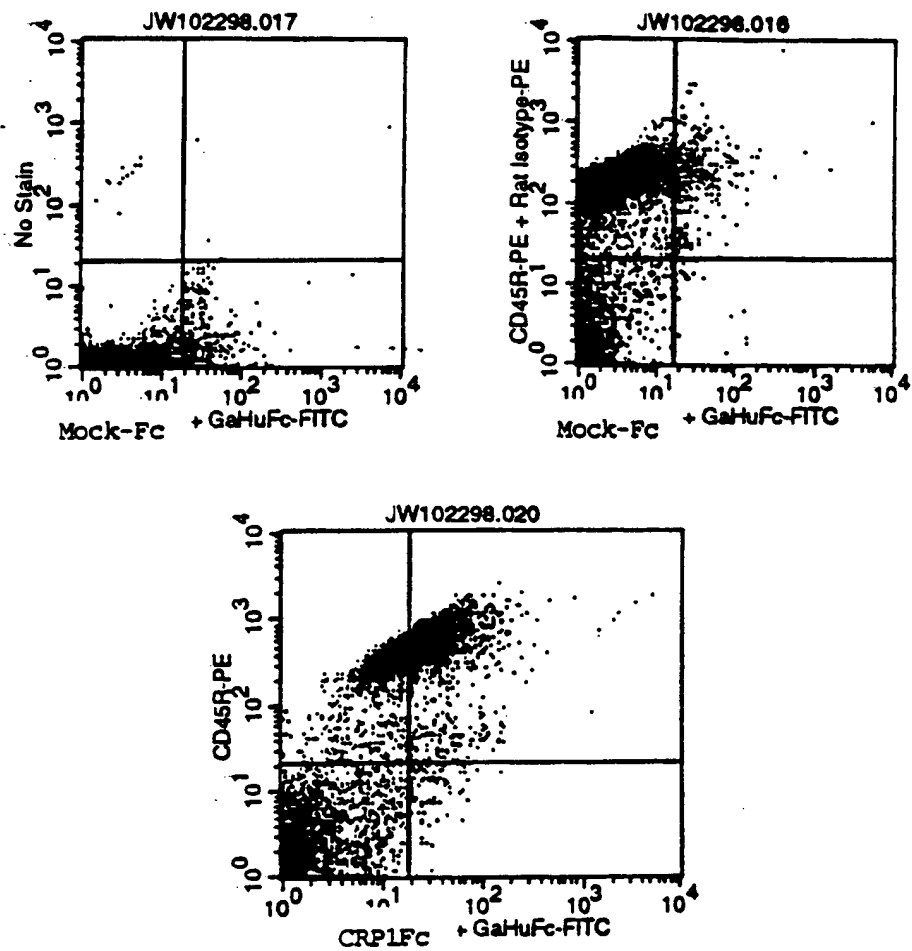
Figure 6
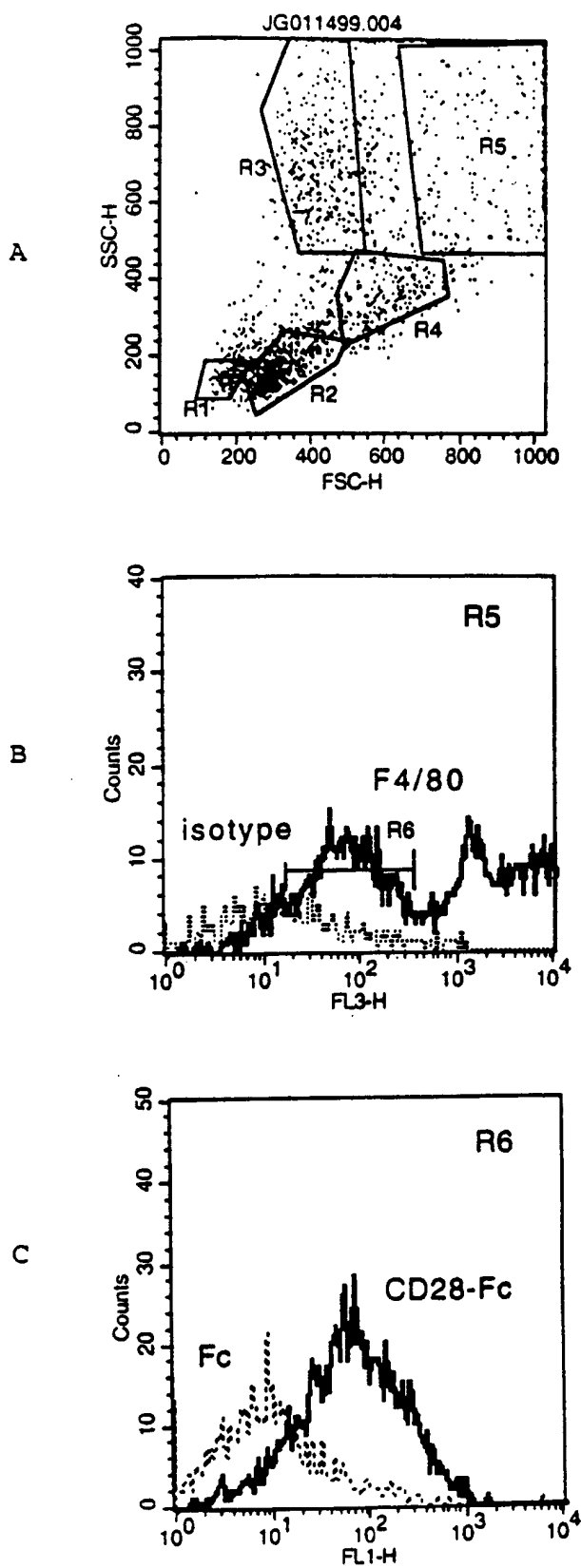
Figure 7

Figure 8

FIGURE 9



Con A stimulation of T-cells regulated
by B7RP1-Fc fusion protein

Figure 10



A  Lymph node-control#10

B  LN-WX11#40

C  LN closeup-control#10

D  LN closeup-WX11#40

E  Spleen-control#10

F  Spleen-WX11#6

G  Ileum-control#25

H  Ileum-WX11#32

Figure 11



A    Control#5-H&E

B    WX11#33

C    Control-B220 (B cells)

D    WX11-B220

E    Control-CD3 (T cells)

F    WX11-CD3
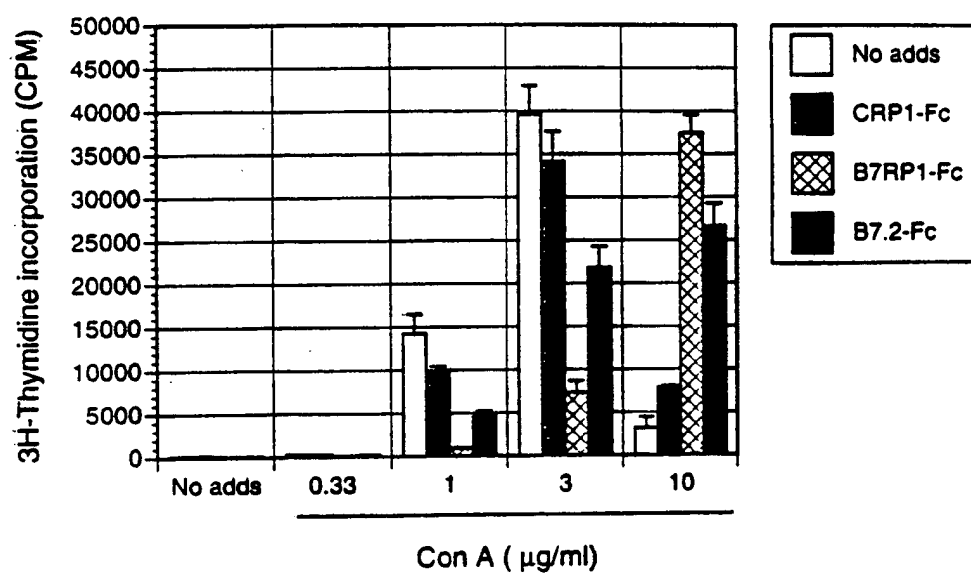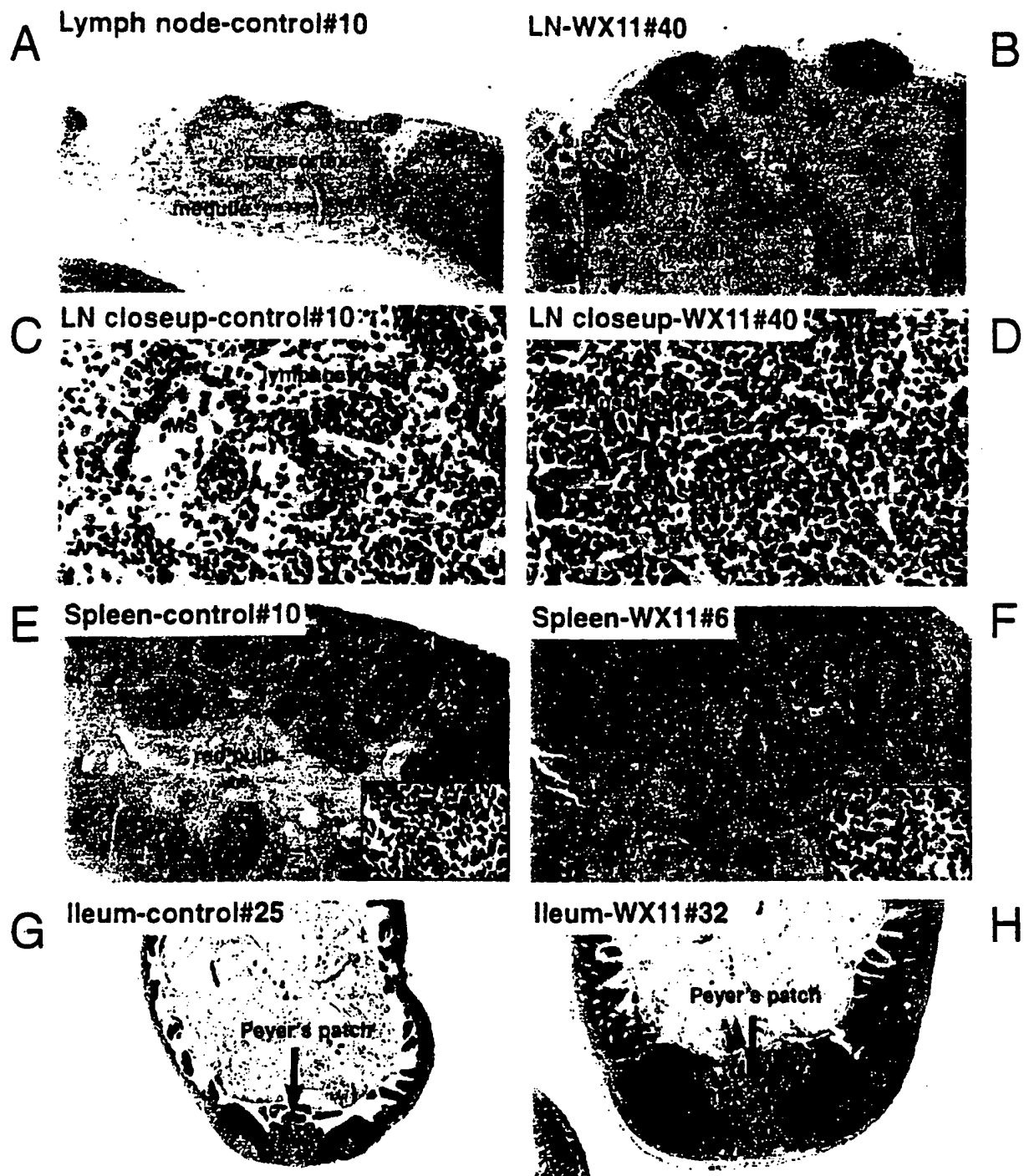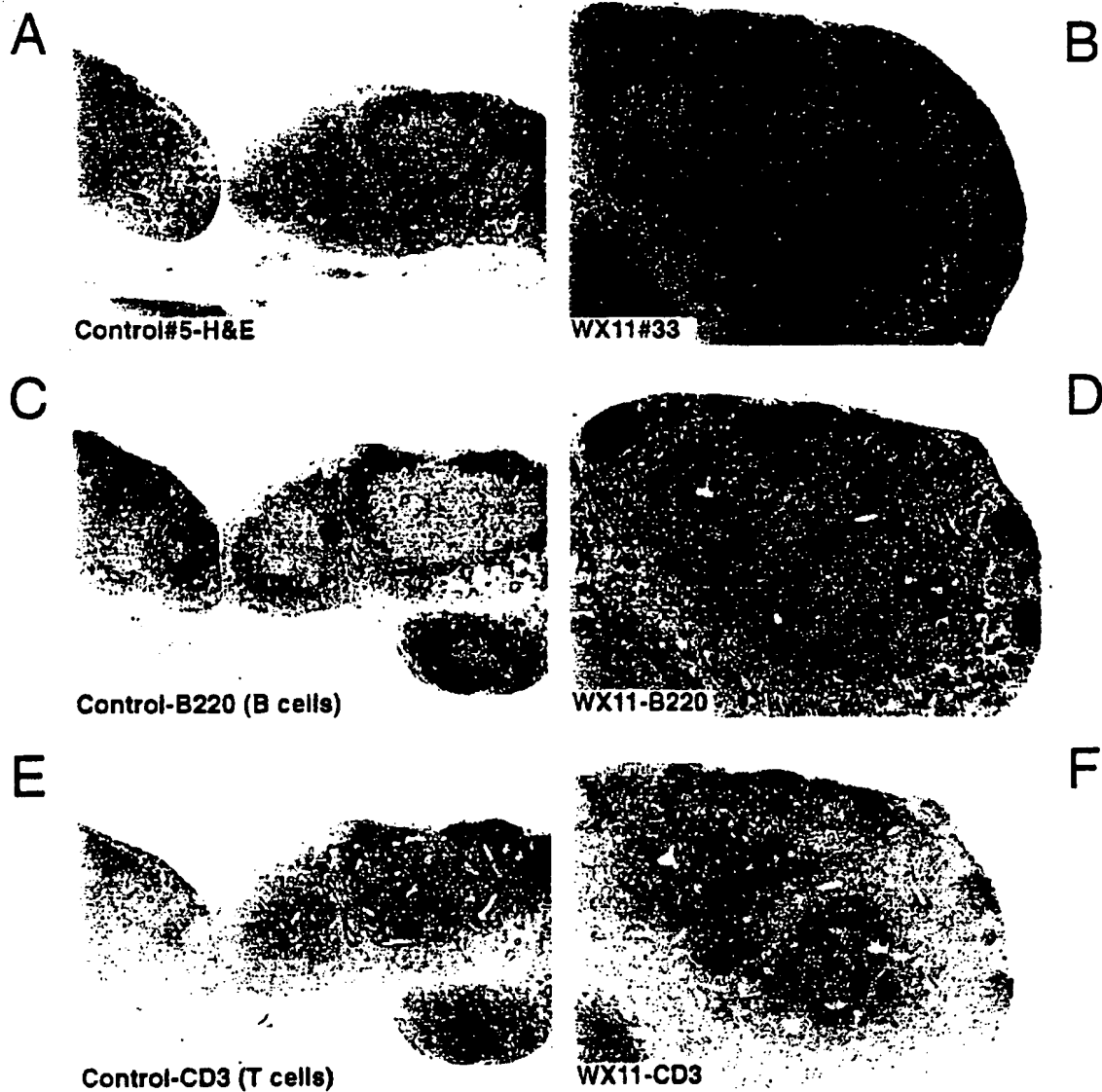
Figure 12A.

```
GCTGGTACGCCTGCAGGTACCGGTCCGGAATTCCCGGGTCGACCCACGCGTCCGCCCACGCG    -138
TCCGCGGGAGCGCAGTTAGAGCCGATCTCCCGCGCCCCGAGGTTGCTCCTCTCCGAGGTCTC    -76
CCGCGGCCCAAGTTCTCCGCGCCCCGAGGTCTCCGCGCCCCGAGGTCTCCGCGGCCCGAGGT    -14
CTCCGCCCGCACC                                                     -1
```

```
ATG CGG CTG GGC AGT CCT GGA CTG CTC TTC CTG CTC TTC AGC AGC        45
M   R   L   G   S   P   G   L   L   F   L   L   F   S   S
                    5             10                15
```

```
CTT CGA GCT GAT ACT CAG GAG AAG GAA GTC AGA GCG ATG GTA GGC        90
L   R   A   *D  *T  *Q  *E  K   *E  V   R   A   *M  V   G
                    20              25                30
```

```
AGC GAC GTG GAG CTC AGC TGC GCT TGC CCT GAA GGA AGC CGT TTT        135
S   D   V   E   L   S   C   A   C   P   E   G   S   R   F
                    35              40                45
```

```
GAT TTA AAT GAT GTT TAC GTA TAT TGG CAA ACC AGT GAG TCG AAA        180
D   L   N   D   V   Y   V   Y   W   Q   T   S   E   S   K
                    50              55                60
```

```
ACC GTG GTG ACC TAC CAC ATC CCA CAG AAC AGC TCC TTG GAA AAC        225
T   V   V   T   Y   H   I   P   Q   N   S   S   L   E   N
                    65              70                75
```

```
GTG GAC AGC CGC TAC CGG AAC CGA GCC CTG ATG TCA CCG GCC GGC        270
V   D   S   R   Y   R   N   R   A   L   M   S   P   A   G
                    80              85                90
```

```
ATG CTG CGG GGC GAC TTC TCC CTG CGC TTG TTC AAC GTC ACC CCC        315
M   L   R   G   D   F   S   L   R   L   F   N   V   T   P
                    95              100               105
```

```
CAG GAC GAG CAG AAG TTT CAC TGC CTG GTG TTG AGC CAA TCC CTG        360
Q   D   E   Q   K   F   H   C   L   V   L   S   Q   S   L
                    110             115               120
```

```
GGA TTC CAG GAG GTT TTG AGC GTT GAG GTT ACA CTG CAT GTG GCA        405
G   F   Q   E   V   L   S   V   E   V   T   L   H   V   A
                    125             130               135
```

```
GCA AAC TTC AGC GTG CCC GTC GTC AGC GCC CCC CAC AGC CCC TCC        450
A   N   F   S   V   P   V   V   S   A   P   H   S   P   S
                    140               145               150
```

```
CAG GAT GAG CTC ACC TTC ACG TGT ACA TCC ATA AAC GGC TAC CCC        495
Q   D   E   L   T   F   T   C   T   S   I   N   G   Y   P
                    155             160               165
```

```
AGG CCC AAC GTG TAC TGG ATC AAT AAG ACG GAC AAC AGC CTG CTG        540
R   P   N   V   Y   W   I   N   K   T   D   N   S   L   L
                    170             175               180
```

```
GAC CAG GCT CTG CAG AAT GAC ACC GTC TTC TTG AAC ATG CGG GGC        585
D   Q   A   L   Q   N   D   T   V   F   L   N   M   R   G
                    185             190               195
```

```
TTG TAT GAC GTG GTC AGC GTG CTG AGG ATC GCA CGG ACC CCC AGC        630
L   Y   D   V   V   S   V   L   R   I   A   R   T   P   S
                    200             205               210
```

```
GTG AAC ATT GGC TGC TGC ATA GAG AAC GTG CTT CTG CAG CAG AAC        675
V   N   I   G   C   C   I   E   N   V   L   L   Q   Q   N
                    215             220               225
```

```
CTG ACT GTC GGC AGC CAG ACA GGA AAT GAC ATC GGA GAG AGA GAC          720
L   T   V   G   S   Q   T   G   N   D   I   G.  E   R   D
                230                     235                 240

AAG ATC ACA GAG AAT CCA GTC AGT ACC GGC GAG AAA AAC GCG GCC          765
K   I   T   E   N   P   V   S · T   G   E   K   N   A   A
                245                     250                 255

ACG TGG AGC ATC CTG GCT GTC CTG TGC CTG CTT GTG GTC GTG GCG          810
T   W   S   I   L   A   V   L   C   L   L   V   V   V   A
                260                     265                 270

GTG GCC ATA GGC TGG GTG TGC AGG GAC CGA TGC CTC CAA CAC AGC          855
V   A   I   G   W   V   C   R   D   R   C   L   Q   H   S
                275                     280                 285

TAT GCA GGT GCC TGG GCT GTG AGT CCG GAG ACA GAG CTC ACT GGC          900
Y   A   G   A   W   A   V   S   P   E   T.  E   L   T.  G
                                                            300

CAC GTT TGA                                                          909
H   V   STOP
        302

CCGGAGCTCACCGCCCAGAGCGTGGACAGGGCTTCCGTGAGACGCCACCGTGAGAGGCCAGG      971
TGGCAGCTTGAGCATGGACTCCCAGACTGCAGGGGAGCACTTGGGGCAGCCCCCAGAAGGAC     1033
CACTGCTGGATCCCAGGGAGAACCTGCTGGCGTTGGCTGTGATCCTGGAATGAGGCCCTTTC     1095
```

Figure 12B.

```
human      MRLGSP----  ----------  ---------G  L-LF-LLFSS  LRADTQEKEV      25
mouse      MQLKCPCFVS  LGTRQPVWKK  LHVSSGFFSG  LGLFLLLLSS  LCAASAETEV      50
Consensus  M..L..P....  ..........  .........G  L.LF.LL.SS  L.A...E.EV      50

human      RAMVGSDVEL  SCACPEGSRF  DLNDVYVYWQ  TSESKTVVTY  HIPQNSSLEN      75
mouse      GAMVGSNVVL  SCIDPHRRHF  NLSGLYVYWQ  IENPEVSVTY  YLPYKSPGIN     100
Consensus  .AMVGS.V.L  SC..P....F  .L...YVYWQ  .......VTY  ..P..S...N     100

human      VDSRYRNRAL  MSPAGMLRGD  FSLRLFNVTP  QDEQKFHCLV  LSQ-SLGFQE     124
mouse      VDSSYKNRGH  LSLDSMKQGN  FSLYLKNVTP  QDTQEFTCRV  FMNTATELVK     150
Consensus  VDS.Y.NR..  .S...M..G.  FSL.L.NVTP  QD.Q.F.C.V  ..........     150

human      VLSVEVTLHV  AANFSVPVVS  APHSPSQ-DE  LTFTCTSING  YPRPNVYWIN     173
mouse      ILEEVVRLRV  AANFSTPVIS  TSDSSNPGQE  RTYTCMSKNG  YPEPNLYWIN     200
Consensus  .L...V.L.V  AANFS.PV.S  ...S.....E  .T.TC.S.NG  YP.PN.YWIN     200

human      KTDNSLLDQA  LQNDTVFLNM  RGLYDVVSVL  RIARTPSVNI  GCCIENVLLQ     223
mouse      TTDNSLIDTA  LQNNTVYLNK  LGLYDVISTL  RLPWTSRGDV  LCCVENVALH     250
Consensus  .TDNSL.D.A  LQN.TV.LN.  .GLYDV.S.L  R...T.....  .CC.ENV.L.     250

human      QNLTVGSQTG  NDIGERDKIT  ENPVSTGEKN  AATWSILAVL  CLLVVVAVAI     273
mouse      QNITSISQAE  SFTGNNTKNP  QETHNNELKV  LV--PVLAVL  AAAAFVSFII     298
Consensus  QN.T..SQ..  ...G...K..  ........K.  .:....LAVL  .....V...I     300

human      GWVCRDRCLQ  HSYAGAWAVS  PETELTGHV                               302
mouse      YR--RTR-PH  RSYTGPKTVQ  LE--LTDHA                               322
Consensus  ....R.R...  .SY.G...V.  .E..LT.H.                               329
```

Figure 13A

```
AACAATTTCACACAGGAAACAGCTATGACCATGATTACGCCAAGCTCTAATACGA        -111
CTCACTATAGGGAAAGCTGGTACGCCTGCAGGTACCGGTCCGGAATTCCCGGGTC        -56
GACCCACGCGTCCGTGAACACTGAACGCGAGGACTGTTAACTGTTTCTGGCAAAC        -1
```

```
ATG AAG TCA GGC CTC TGG TAT TTC TTT CTC TTC TGC TTG CGC ATT 45
 M   K   S   G   L   W   Y   F   F   L   F   C   L   R   I
                 5               10                  15
```

```
AAA GTT TTA ACA GGA GAA ATC AAT GGT TCT GCC AAT TAT GAG ATG        90
 K   V   L   T  *G  *E   I   N   G   S   A   N   Y   E   M
                 20                  25                  30
```

```
TTT ATA TTT CAC AAC GGA GGT GTA CAA ATT TTA TGC AAA TAT CCT        135
 F   I   F   H   N   G   G   V   Q   I   L   C   K   Y   P
                 35                  40                  45
```

```
GAC ATT GTC CAG CAA TTT AAA ATG CAG TTG CTG AAA GGG GGG CAA        180
 D   I   V   Q   Q   F   K   M   Q   L   L   K   G   G   Q
                 50                  55                  60
```

```
ATA CTC TGC GAT CTC ACT AAG ACA AAA GGA AGT GGA AAC ACA GTG        225
 I   L   C   D   L   T   K   T   K   G   S   G   N   T   V
                 65                  70                  75
```

```
TCC ATT AAG AGT CTG AAA TTC TGC CAT TCT CAG TTA TCC AAC AAC        270
 S   I   K   S   L   K   F   C   H   S   Q   L   S   N   N
                 80                  85                  90
```

```
AGT GTC TCT TTT TTT CTA TAC AAC TTG GAC CAT TCT CAT GCC AAC        315
 S   V   S   F   F   L   Y   N   L   D   H   S   H   A   N
                 95                 100                 105
```

```
TAT TAC TTC TGC AAC CTA TCA ATT TTT GAT CCT CCT CCT TTT AAA        360
 Y   Y   F   C   N   L   S   I   F   D   P   P   P   F   K
                110                 115                 120
```

```
GTA ACT CTT ACA GGA GGA TAT TTG CAT ATT TAT GAA TCA CAA CTT        405
 V   T   L   T   G   G   Y   L   H   I   Y   E   S   Q   L
                125                 130                 135
```

```
TGT TGC CAG CTG AAG TTC TGG TTA CCC ATA GGA TGT GCA GCC TTT        450
 C   C   Q   L   K   F   W   L   P   I   G   C   A   A   F
                140                 145                 150
```

```
GTT GTA GTC TGC ATT TTG GGA TGC ATA CTT ATT TGT TGG CTT ACA        495
 V   V   V   C   I   L   G   C   I   L   I   C   W   L   T
                155                 160                 165
```

```
AAA AAG AAG TAT TCA TCC AGT GTG CAC GAC CCT AAC GGT GAA TAC        540
 K   K   K   Y   S   S   S   V   H   D   P   N   G   E   Y
                170                 175                 180
```

```
ATG TTC ATG AGA GCA GTG AAC ACA GCC AAA AAA TCT AGA CTC ACA        585
 M   F   M   R   A   V   N   T   A   K   K   S   R   L   T
                185                 190                 195
```

```
                                                               600
GAT GTG ACC CTA TAA
 D   V   T   L   STOP
                199
```

```
                                                               655
TATGGAACTCTGGCACCCAGGCATGAAGCACGTTGGCCAGTTTTCCTCAACTTGA
AGTGCAAGATTCTCTTATTTCCGGGACCACGGAGAGTCTGACTTAACTACATACA        710
```

```
TCTTCTGCTGGTGTTTTGTTCAATCTGGAAGAATGACTGTATCAGTCAATGGGGA          765
TTTTAACAGACTGCCTTGGTACTGCCGAGTCCTCTCAAAACAAACACCCTCTTGC          820
AACCAGCTTTGGAGAAAGCCCAGCTCCTGTGTGCTCACTGGGAGTGGAATCCCTG          875
TCTCCACATCTGCTCCTAGCAGTGCATCAGCCAGTAAAACAAACACATTTACAAG          930
AAAAATGTTTTAAAGATGCCAGGGGTACTGAATCTGCAAAGCAAATGAGCAGCCA          985
AGGACCAGCATCTGTCCGCATTTCACTATCATACTACCTCTTCTTTCTGTAGGGA          1040
TGAGAATTCCTCTTTTAATCAGTCAAGGGAGATGCTTCAAAGCTGGAGCTATTTT          1095
ATTTCTGAGATGTTGATGTGAACTGTACATTAGTACATACTCAGTACTCTCCTTC          1150
AATTGCTGAACCCCAGTTGACCATTTTACCAAGACTTTAGATGCTTTCTTGTGCC          1205
```

Figure 13B

```
hCRP1      MKSGLWYFFLFCLRIKVLTGEINGSANYEMFIFHNGGVQILCKYPDIVQQ      50
           ||         |.||    |:.|||||||||.: || |||||||| ||||: |||
mCRP1      MKPYFCRVFVFCFLIRLLTGEINGSADHRMFSFHNGGVQISCKYPETVQQ      50

hCRP1      FKMQLLKGGQILCDLTKTKGSGNTVSIKSLKFCHSQLSNNSVSFFLYNLD     100
           ||.|  :   ::|||||||||||||| ||||.   ||   |||||||||| | |
mCRP1   ·· LKMRLFREREVLCELTKTKGSGNAVSIKNPMLCLYHLSNNSVSFFLNNPD     100

hCRP1      HSHANYYFCNLSIFDPPPFKV.TLTGGYLHIYESQLCCQLKFWLPIGCAA     149
           |  .|||||||||||||||||.   |.|||||||||||||||| |||:||||
mCRP1·     SSQGSYYFCSLSIFDPPPFQERNLSGGYLHIYESQLCCQLKLWLPVGCAA     150

hCRP1      FVVVCILGCILICWLTKKKYSSSVHDPNGEYMFMRAVNTAKKSRLTDVTL     199
           ||||  : ||||| |  .|||| ||||||| |||||  |||| ||||| ||
mCRP1      FVVVLLFGCILIIWFSKKKYGSSVHDPNSEYMFMAAVNTNKKSRLAGVTS     200
```

Figure 14

Figure 15

A.



B.



Figure 16

Figure 20



**Effect of B7RP1-Fc on Meth A Tumor Growth in Balb-C Mice**
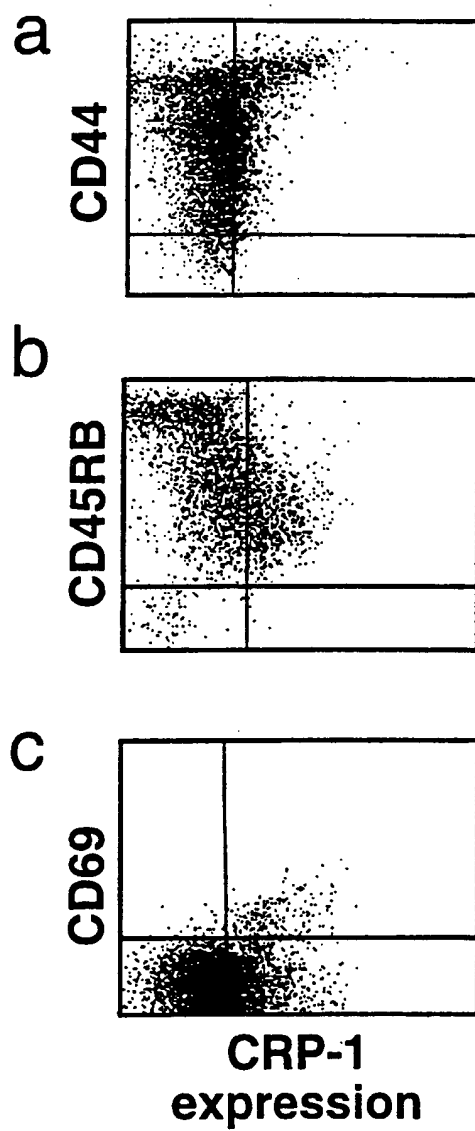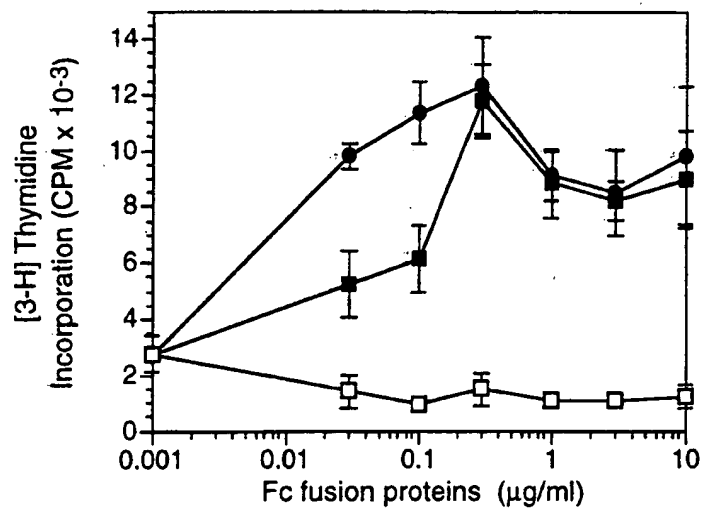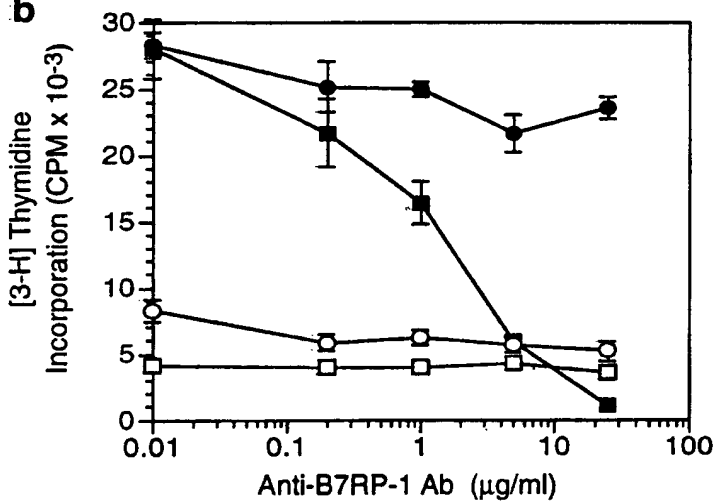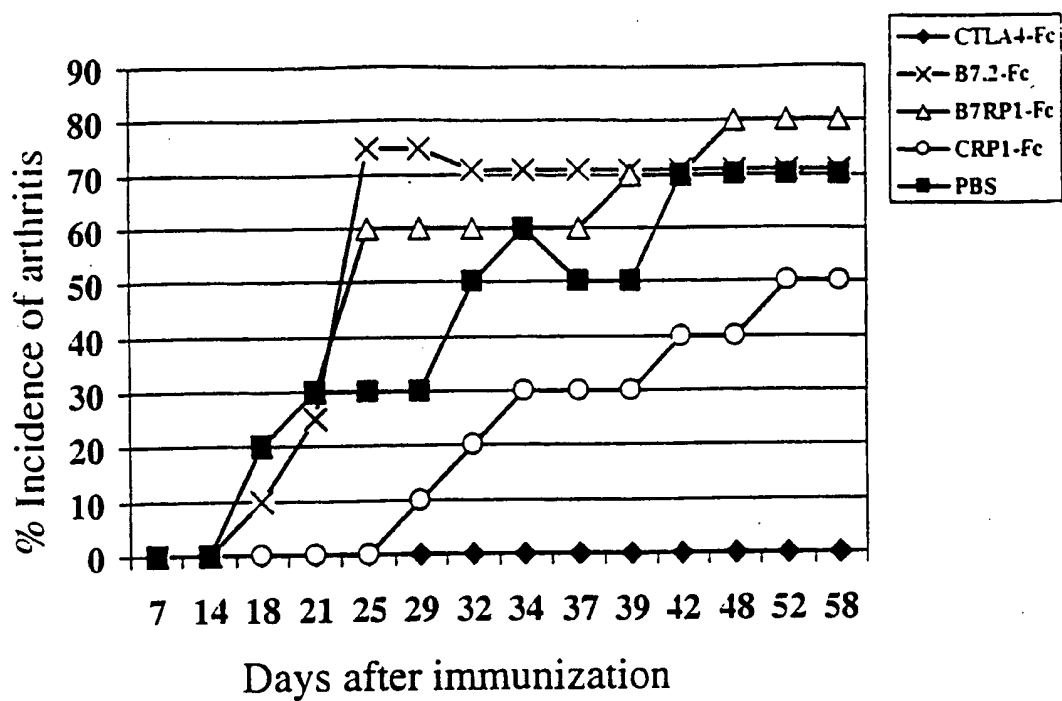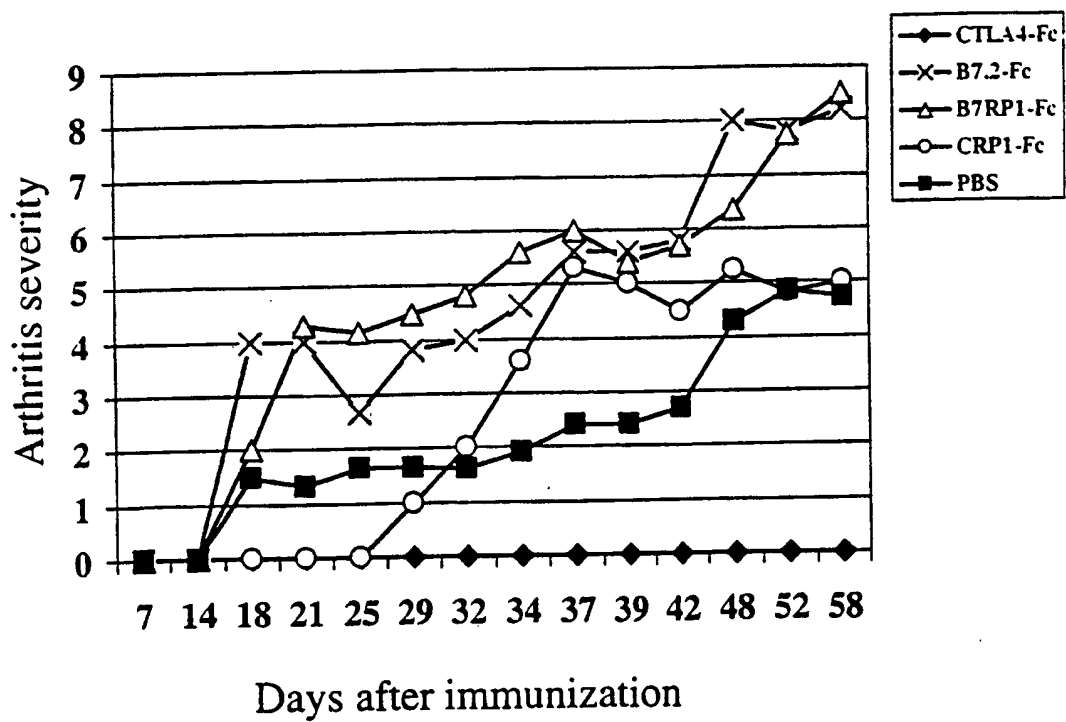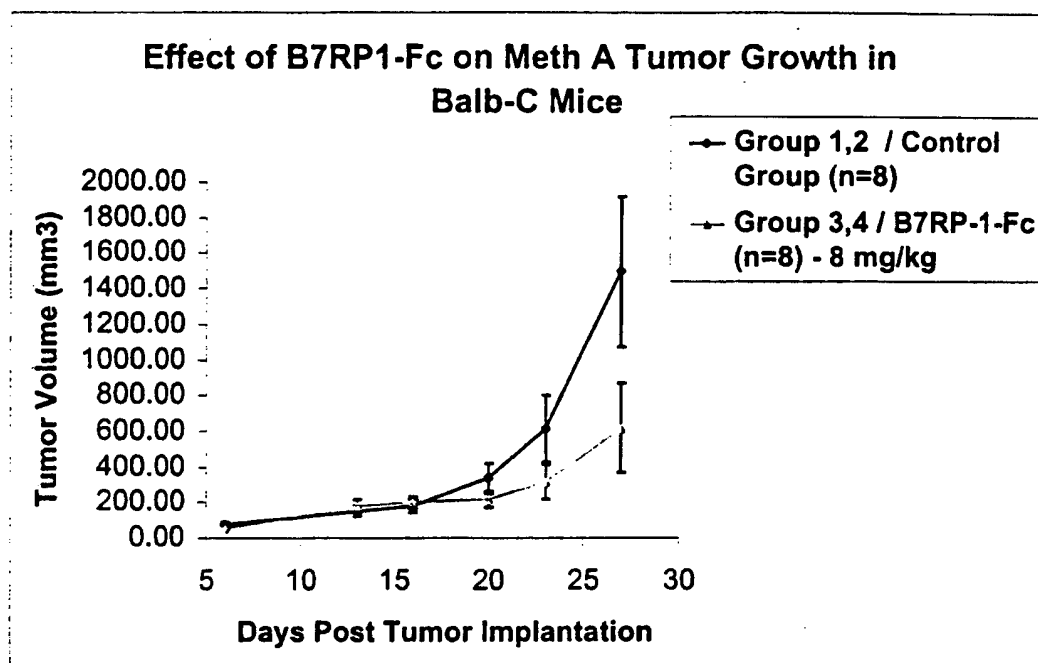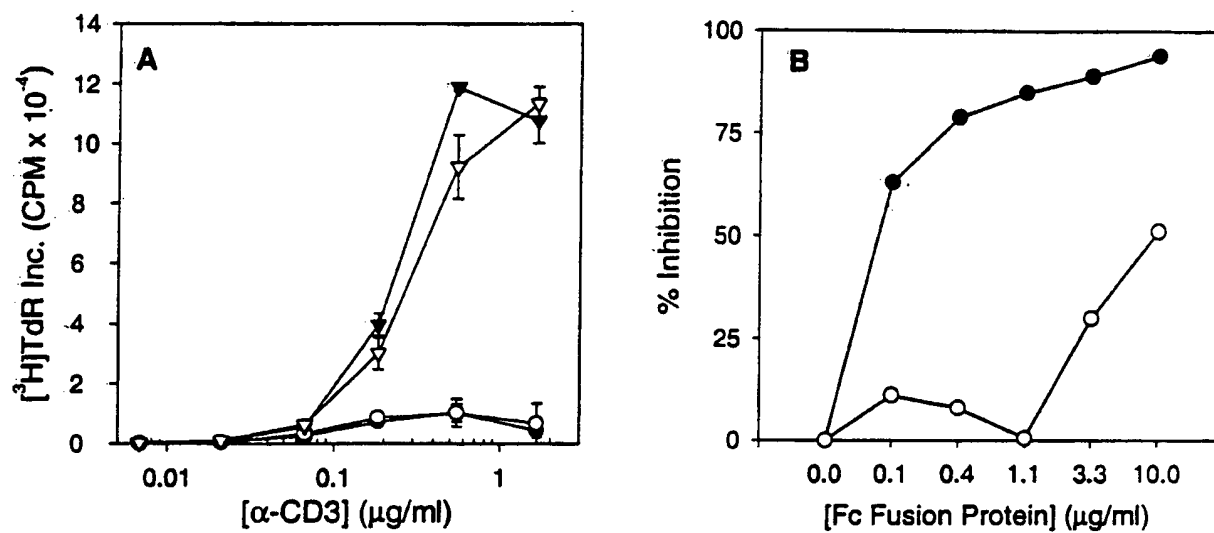
Figure 21A/B.
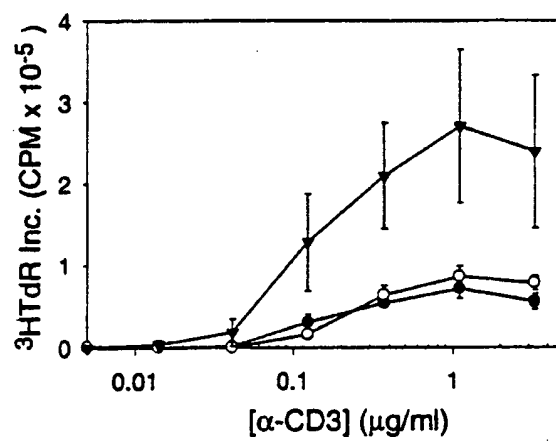
Figure 21C.

Figure 21D
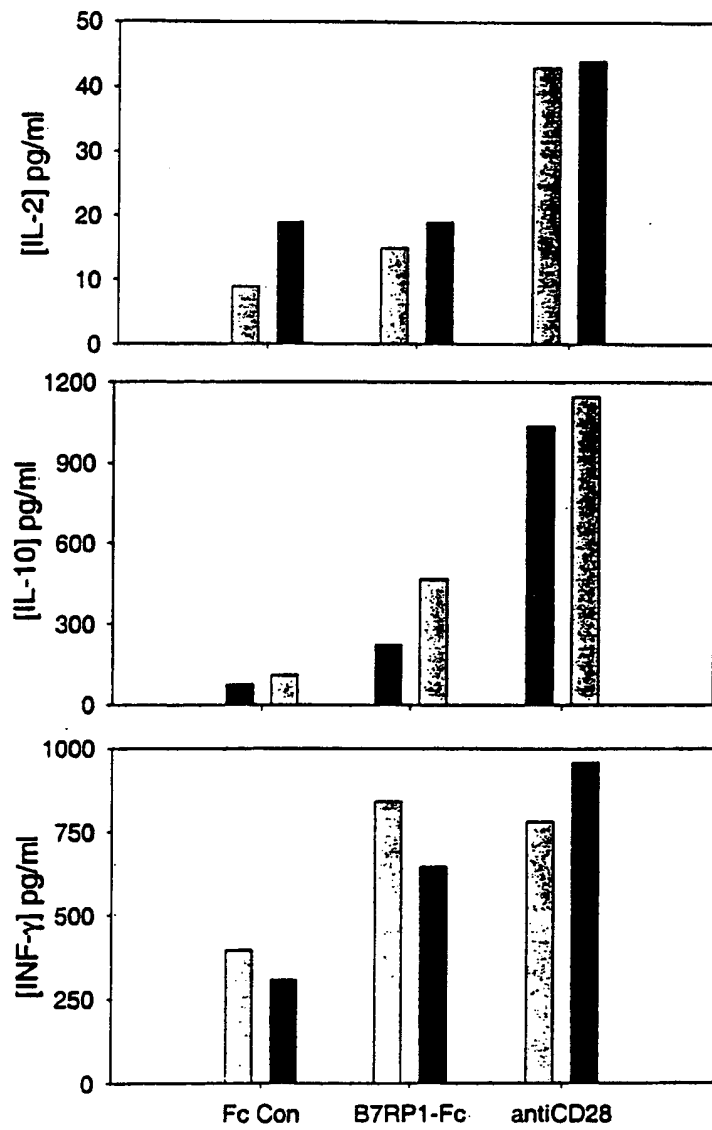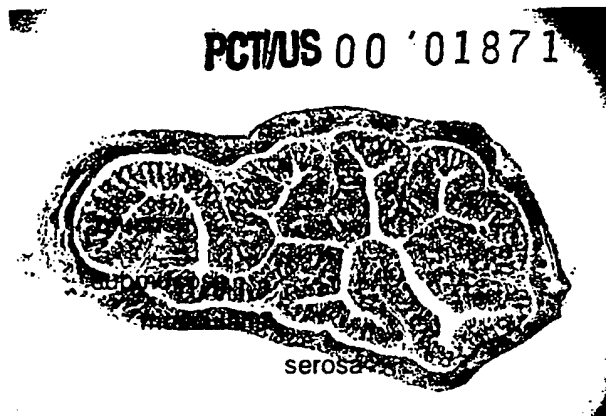
A. Control mouse#53F:Prox. colon 40X
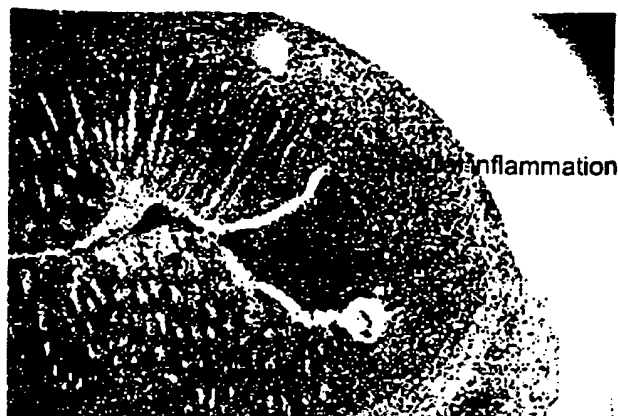
B. Mouse#111F:Prox. colon 40X

C. Mouse#111F: Prox. colon 20X

D. Mouse#111F: closeup of mucosa 100X

E. Mouse#112F: Giant cell, submucosa
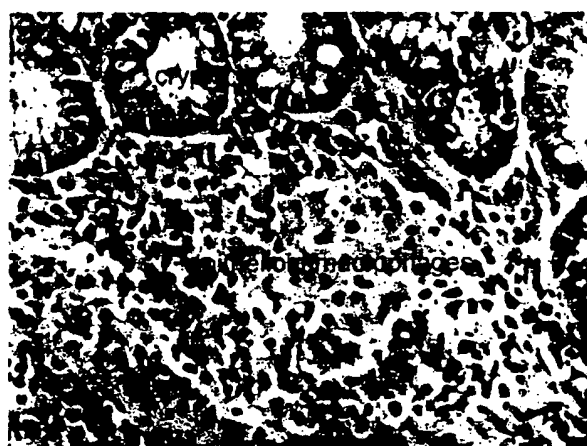
F. Mouse#112F:epith lioid macrophages

Figure 17
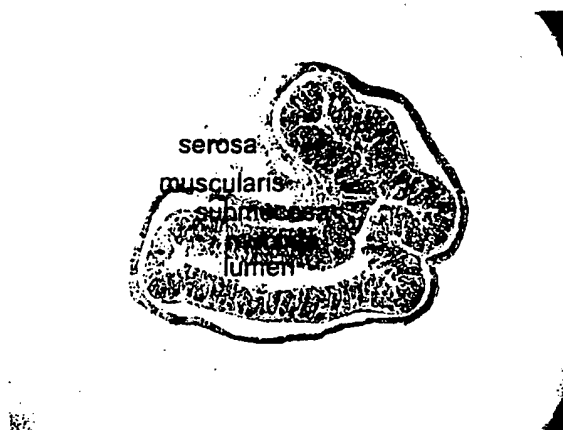
A. Control mouse#53F:Distal colon, 40X

B. mouse#111F:Distal colitis, 40X
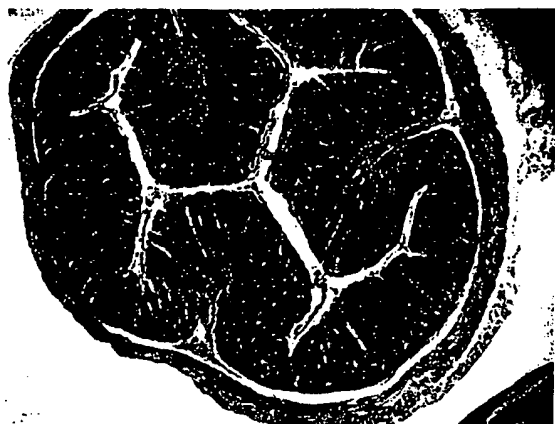
C. mouse#55M:Distal colitis, 40X

D. mouse#112F:Distal colon, 40X

E. mouse#112:CD3+ T-cells, 40X
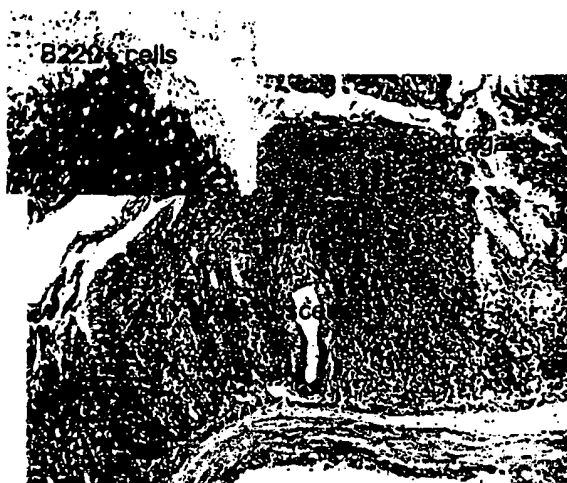
F. mouse#112:closeup, 100X

Figure 18

A. Control mouse#53F:duodenum, 40X

B. Mouse#51F:duodenum, 40X
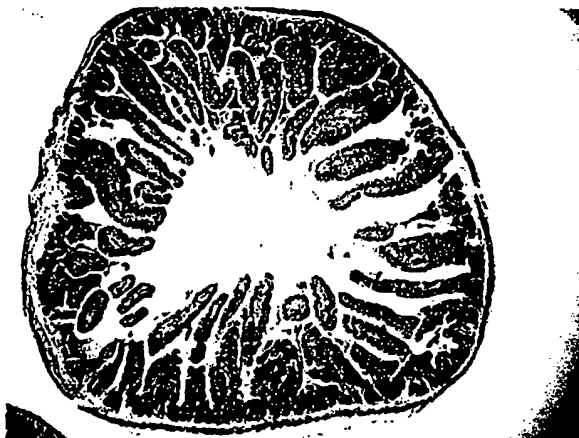
C. Control mouse#53F:jejunum, 40X
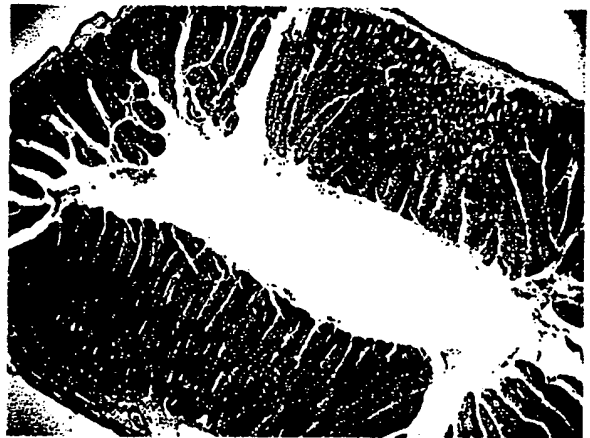
D. Mouse#51F:jejunal hyperplasia, 40X
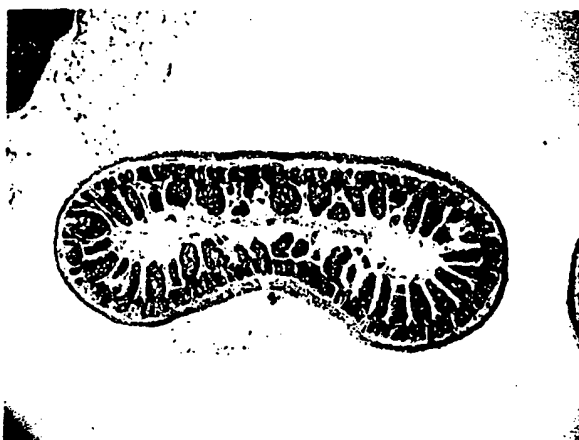
E. Control mouse#53F:ileum, 40X
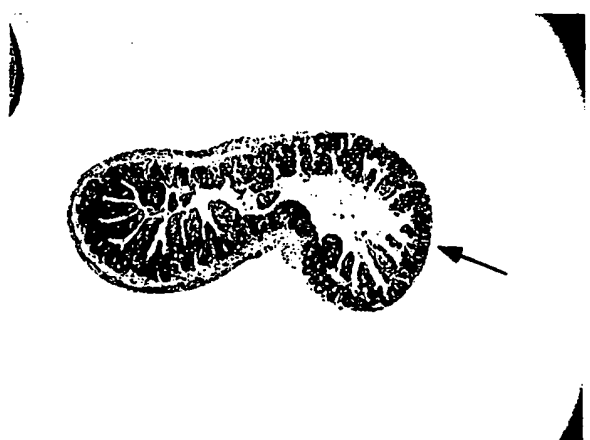
F. Mouse#231M:Ileal atrophy, 40X

Figure 19

SEQUENCE LISTING

<110> AMGEN INC.

<120> NOVEL POLYPEPTIDES INVOLVED IN IMMUNE RESPONSE

<130> A-579A

<140> 09/264,527
<141> 1999-03-08

<160> 35

<170> PatentIn Ver. 2.1

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  1               5                  10                  15

ctt tta aca gga gaa atc aat ggc tcg gcc gat cat agg atg ttt tca      96
Leu Leu Thr Gly Glu Ile Asn Gly Ser Ala Asp His Arg Met Phe Ser
             20                  25                  30

ttt cac aat gga ggt gta cag att tct tgt aaa tac cct gag act gtc     144
Phe His Asn Gly Gly Val Gln Ile Ser Cys Lys Tyr Pro Glu Thr Val
         35                  40                  45

cag cag tta aaa atg cga ttg ttc aga gag aga gaa gtc ctc tgc gaa     192
Gln Gln Leu Lys Met Arg Leu Phe Arg Glu Arg Glu Val Leu Cys Glu
     50                  55                  60

ctc acc aag acc aag gga agc gga aat gcg gtg tcc atc aag aat cca     240
Leu Thr Lys Thr Lys Gly Ser Gly Asn Ala Val Ser Ile Lys Asn Pro
 65                  70                  75                  80

atg ctc tgt cta tat cat ctg tca aac aac agc gtc tct ttt ttc cta     288
Met Leu Cys Leu Tyr His Leu Ser Asn Asn Ser Val Ser Phe Phe Leu
             85                  90                  95

aac aac cca gac agc tcc cag gga agc tat tac ttc tgc agc ctg tcc     336
Asn Asn Pro Asp Ser Ser Gln Gly Ser Tyr Tyr Phe Cys Ser Leu Ser
            100                 105                 110

att ttt gac cca cct cct ttt caa gaa agg aac ctt agt gga gga tat     384
Ile Phe Asp Pro Pro Pro Phe Gln Glu Arg Asn Leu Ser Gly Gly Tyr
        115                 120                 125

ttg cat att tat gaa tcc cag ctc tgc tgc cag ctg aag ctc tgg cta     432
Leu His Ile Tyr Glu Ser Gln Leu Cys Cys Gln Leu Lys Leu Trp Leu
        130                 135                 140

ccc gta ggg tgt gca gct ttc gtt gtg gta ctc ctt ttt gga tgc ata     480
Pro Val Gly Cys Ala Ala Phe Val Val Val Leu Leu Phe Gly Cys Ile
145                 150                 155                 160
```

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ctt atc atc tgg ttt tca aaa aag aaa tac gga tcc agt gtg cat gac   528
Leu Ile Ile Trp Phe Ser Lys Lys Lys Tyr Gly Ser Ser Val His Asp
            165             170                 175

cct aat agt gaa tac atg ttc atg gcg gca gtc aac aca aac aaa aag   576
Pro Asn Ser Glu Tyr Met Phe Met Ala Ala Val Asn Thr Asn Lys Lys
            180             185                 190

tct aga ctt gca ggt gtg acc tca                                   600
Ser Arg Leu Ala Gly Val Thr Ser
            195             200
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Leu Leu Thr Gly Glu Ile Asn Gly Ser Ala Asp His Arg Met Phe Ser
            20              25                  30

Phe His Asn Gly Gly Val Gln Ile Ser Cys Lys Tyr Pro Glu Thr Val
        35              40                  45

Gln Gln Leu Lys Met Arg Leu Phe Arg Glu Arg Glu Val Leu Cys Glu
    50              55                  60

Leu Thr Lys Thr Lys Gly Ser Gly Asn Ala Val Ser Ile Lys Asn Pro
65              70                  75                  80

Met Leu Cys Leu Tyr His Leu Ser Asn Asn Ser Val Ser Phe Phe Leu
                85              90                  95

Asn Asn Pro Asp Ser Ser Gln Gly Ser Tyr Tyr Phe Cys Ser Leu Ser
            100             105                 110

Ile Phe Asp Pro Pro Pro Phe Gln Glu Arg Asn Leu Ser Gly Gly Tyr
        115             120                 125

Leu His Ile Tyr Glu Ser Gln Leu Cys Cys Gln Leu Lys Leu Trp Leu
    130             135                 140

Pro Val Gly Cys Ala Ala Phe Val Val Val Leu Leu Phe Gly Cys Ile
145             150                 155                 160

Leu Ile Ile Trp Phe Ser Lys Lys Lys Tyr Gly Ser Ser Val His Asp
            165             170                 175

Pro Asn Ser Glu Tyr Met Phe Met Ala Ala Val Asn Thr Asn Lys Lys
            180             185                 190

Ser Arg Leu Ala Gly Val Thr Ser
            195             200
```

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<212> PRT
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<213> mouse

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 1               5              10                  15

Leu Leu Thr Gly Glu Ile Asn Gly Ser Ala Asp His Arg Met Phe Ser
             20                  25                  30

Phe His Asn Gly Gly Val Gln Ile Ser Cys Lys Tyr Pro Glu Thr Val
         35                  40                  45

Gln Gln Leu Lys Met Arg Leu Phe Arg Glu Arg Glu Val Leu Cys Glu
     50                  55                  60

Leu Thr Lys Thr Lys Gly Ser Gly Asn Ala Val Ser Ile Lys Asn Pro
 65                  70                  75                  80

Met Leu Cys Leu Tyr His Leu Ser Asn Asn Ser Val Ser Phe Phe Leu
                 85                  90                  95

Asn Asn Pro Asp Ser Ser Gln Gly Ser Tyr Tyr Phe Cys Ser Leu Ser
            100                 105                 110

Ile Phe Asp Pro Pro Pro Phe Gln Glu Arg Asn Leu Ser Gly Gly Tyr
        115                 120                 125

Leu His Ile Tyr Glu Ser Gln Leu Cys Cys Gln Leu Lys Leu Trp Leu
        130                 135                 140

Pro Val Gly Cys Ala Ala Phe Val Val Val Leu Leu Phe Gly Cys Ile
145                 150                 155                 160

Leu Ile Ile Trp Phe Ser Lys Lys Lys Tyr Gly Ser Ser Val His Asp
                165                 170                 175

Pro Asn Ser Glu Tyr Met Phe Met Ala Ala Val Asn Thr Asn Lys Lys
            180                 185                 190

Ser Arg Leu Ala Gly Val Thr Ser
            195                 200


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             20                  25                  30

Asp Ser Asn Glu Val Ser Leu Ser Cys Arg Tyr Ser Tyr Asn Leu Leu
         35                  40                  45

Ala Lys Glu Phe Arg Ala Ser Leu Tyr Lys Gly Val Asn Ser Asp Val
     50                  55                  60

Glu Val Cys Val Gly Asn Gly Asn Phe Thr Tyr Gln Pro Gln Phe Arg
 65                  70                  75                  80
```

```
Ser Asn Ala Glu Phe Asn Cys Asp Gly Asp Phe Asp Asn Glu Thr Val
                85                  90                  95

Thr Phe Arg Leu Trp Asn Leu His Val Asn His Thr Asp Ile Tyr Phe
            100             105                 110

Cys Lys Ile Glu Phe Met Tyr Pro Pro Pro Tyr Leu Asp Asn Glu Arg
            115             120             125

Ser Asn Gly Thr Ile Ile His Ile Lys Glu Lys His Leu Cys His Thr
    130             135             140

Gln Ser Ser Pro Lys Leu Phe Trp Ala Leu Val Val Val Ala Gly Val
145             150             155                 160

Leu Phe Cys Tyr Gly Leu Leu Val Thr Val Ala Leu Cys Val Ile Trp
            165             170             175

Thr Asn Ser Arg Arg Asn Arg Leu Leu Gln Val Thr Thr Met Asn Met
            180             185             190

Thr Pro Arg Arg Pro Gly Leu Thr Arg Lys Pro Tyr Gln Pro Tyr Ala
            195             200             205

Pro Ala Arg Asp Phe Ala Ala Tyr Arg Pro
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      Oglionucleotide

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            20                  25                  30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        35                  40                  45

Val Xaa Xaa Ser Cys Xaa Tyr Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
    50                  55                  60

Xaa Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa Val Xaa Xaa Cys Xaa
65              70                  75                  80

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
            85                  90                  95

Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Asn Xaa Xaa Val Xaa Phe Xaa Leu
            100             105             110

Xaa Asn Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Tyr Phe Cys Xaa Xaa Xaa
        115                 120             125

Xaa Xaa Xaa Pro Pro Pro Xaa Xaa Xaa Xaa Xaa Xaa Ser Xaa Gly Xaa
    130             135                 140
```

```
Xaa Xaa His Ile Xaa Glu Xaa Xaa Leu Cys Xaa Xaa Xaa Xaa Xaa Xaa
145             150             155                         160

Lys Leu Xaa Trp Xaa Leu Xaa Val Xaa Xaa Xaa Xaa Xaa Phe Xaa Xaa
                165             170                 175

Xaa Xaa Leu Leu Xaa Xaa Xaa Xaa Leu Xaa Xaa Ile Trp Xaa Xaa Xaa
            180             185             190

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Pro Xaa
            195             200             205

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Arg
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Xaa Xaa Ala Xaa Xaa Xaa Xaa Xaa Xaa Xaa
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Met Gln Leu Lys Cys Pro Cys Phe Val Ser Leu Gly Thr Arg Gln Pro
 1               5                  10                  15

gtt tgg aag aag ctc cat gtt tct agc ggg ttc ttt tct ggt ctt ggt      96
Val Trp Lys Lys Leu His Val Ser Ser Gly Phe Phe Ser Gly Leu Gly
                20                  25                  30

ctg ttc ttg ctg ctg ttg agc agc ctc tgt gct gcc tct gca gag act     144
Leu Phe Leu Leu Leu Leu Ser Ser Leu Cys Ala Ala Ser Ala Glu Thr
            35                  40                  45

gaa gtc ggt gca atg gtg ggc agc aat gtg gtg ctc agc tgc att gac     192
Glu Val Gly Ala Met Val Gly Ser Asn Val Val Leu Ser Cys Ile Asp
        50                  55                  60

ccc cac aga cgc cat ttc aac ttg agt ggt ctg tat gtc tat tgg caa     240
Pro His Arg Arg His Phe Asn Leu Ser Gly Leu Tyr Val Tyr Trp Gln
65                  70                  75                  80

atc gaa aac cca gaa gtt tcg gtg act tac tac ctg cct tac aag tct     288
Ile Glu Asn Pro Glu Val Ser Val Thr Tyr Tyr Leu Pro Tyr Lys Ser
                85                  90                  95

cca ggg atc aat gtg gac agt tcc tac aag aac agg ggc cat ctg tcc     336
Pro Gly Ile Asn Val Asp Ser Ser Tyr Lys Asn Arg Gly His Leu Ser
                100                 105                 110

ctg gac tcc atg aag cag ggt aac ttc tct ctg tac ctg aag aat gtc     384
Leu Asp Ser Met Lys Gln Gly Asn Phe Ser Leu Tyr Leu Lys Asn Val
            115                 120                 125

acc cct cag gat acc cag gag ttc aca tgc cgg gta ttt atg aat aca     432
Thr Pro Gln Asp Thr Gln Glu Phe Thr Cys Arg Val Phe Met Asn Thr
```

```
             130                    135                    140

gcc aca gag tta gtc aag atc ttg gaa gag gtg gtc agg ctg cgt gtg    480
Ala Thr Glu Leu Val Lys Ile Leu Glu Glu Val Val Arg Leu Arg Val
145                 150                 155                 160

gca gca aac ttc agt aca cct gtc atc agc acc tct gat agc tcc aac    528
Ala Ala Asn Phe Ser Thr Pro Val Ile Ser Thr Ser Asp Ser Ser Asn
                    165                 170                 175

ccg ggc cag gaa cgt acc tac acc tgc atg tcc aag aat ggc tac cca    576
Pro Gly Gln Glu Arg Thr Tyr Thr Cys Met Ser Lys Asn Gly Tyr Pro
                180                 185                 190

gag ccc aac ctg tat tgg atc aac aca acg gac aat agc cta ata gac    624
Glu Pro Asn Leu Tyr Trp Ile Asn Thr Thr Asp Asn Ser Leu Ile Asp
            195                 200                 205

acg gct ctg cag aat aac act gtc tac ttg aac aag ttg ggc ctg tat    672
Thr Ala Leu Gln Asn Asn Thr Val Tyr Leu Asn Lys Leu Gly Leu Tyr
        210                 215                 220

gat gta atc agc aca tta agg ctc cct tgg aca tct cgt ggg gat gtt    720
Asp Val Ile Ser Thr Leu Arg Leu Pro Trp Thr Ser Arg Gly Asp Val
225                 230                 235                 240

ctg tgc tgc gta gag aat gtg gct ctc cac cag aac atc act agc att    768
Leu Cys Cys Val Glu Asn Val Ala Leu His Gln Asn Ile Thr Ser Ile
                    245                 250                 255

agc cag gca gaa agt ttc act gga aat aac aca aag aac cca cag gaa    816
Ser Gln Ala Glu Ser Phe Thr Gly Asn Asn Thr Lys Asn Pro Gln Glu
                260                 265                 270

acc cac aat aat gag tta aaa gtc ctt gtc ccc gtc ctt gct gta ctg    864
Thr His Asn Asn Glu Leu Lys Val Leu Val Pro Val Leu Ala Val Leu
            275                 280                 285

gcg gca gcg gca ttc gtt tcc ttc atc ata tac aga cgc acg cgt ccc    912
Ala Ala Ala Ala Phe Val Ser Phe Ile Ile Tyr Arg Arg Thr Arg Pro
        290                 295                 300

cac cga agc tat aca gga ccc aag act gta cag ctt gaa ctt aca gac    960
His Arg Ser Tyr Thr Gly Pro Lys Thr Val Gln Leu Glu Leu Thr Asp
305                 310                 315                 320

cac gcc                                                            966
His Ala
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Val Trp Lys Lys Leu His Val Ser Ser Gly Phe Phe Ser Gly Leu Gly
            20                  25                  30

Leu Phe Leu Leu Leu Leu Ser Ser Leu Cys Ala Ala Ser Ala Glu Thr
        35                  40                  45
```

```
Glu Val Gly Ala Met Val Gly Ser Asn Val Val Leu Ser Cys Ile Asp
    50                  55                  60

Pro His Arg Arg His Phe Asn Leu Ser Gly Leu Tyr Val Tyr Trp Gln
65                  70                  75                      80

Ile Glu Asn Pro Glu Val Ser Val Thr Tyr Tyr Leu Pro Tyr Lys Ser
                85                  90                  95

Pro Gly Ile Asn Val Asp Ser Ser Tyr Lys Asn Arg Gly His Leu Ser
            100                 105                 110

Leu Asp Ser Met Lys Gln Gly Asn Phe Ser Leu Tyr Leu Lys Asn Val
        115                 120                 125

Thr Pro Gln Asp Thr Gln Glu Phe Thr Cys Arg Val Phe Met Asn Thr
    130                 135                 140

Ala Thr Glu Leu Val Lys Ile Leu Glu Glu Val Val Arg Leu Arg Val
145                 150                 155                 160

Ala Ala Asn Phe Ser Thr Pro Val Ile Ser Thr Ser Asp Ser Ser Asn
                165                 170                 175

Pro Gly Gln Glu Arg Thr Tyr Thr Cys Met Ser Lys Asn Gly Tyr Pro
            180                 185                 190

Glu Pro Asn Leu Tyr Trp Ile Asn Thr Thr Asp Asn Ser Leu Ile Asp
        195                 200                 205

Thr Ala Leu Gln Asn Asn Thr Val Tyr Leu Asn Lys Leu Gly Leu Tyr
    210                 215                 220

Asp Val Ile Ser Thr Leu Arg Leu Pro Trp Thr Ser Arg Gly Asp Val
225                 230                 235                 240

Leu Cys Cys Val Glu Asn Val Ala Leu His Gln Asn Ile Thr Ser Ile
                245                 250                 255

Ser Gln Ala Glu Ser Phe Thr Gly Asn Asn Thr Lys Asn Pro Gln Glu
            260                 265                 270

Thr His Asn Asn Glu Leu Lys Val Leu Val Pro Val Leu Ala Val Leu
    275                 280                 285

Ala Ala Ala Ala Phe Val Ser Phe Ile Ile Tyr Arg Arg Thr Arg Pro
    290                 295                 300

His Arg Ser Tyr Thr Gly Pro Lys Thr Val Gln Leu Glu Leu Thr Asp
305                 310                 315                 320

His Ala
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<211> 322
<212> PRT
<213> mouse

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```
      1                  5                  10                 15

Val Trp Lys Lys Leu His Val Ser Ser Gly Phe Phe Ser Gly Leu Gly
              20                  25                  30

Leu Phe Leu Leu Leu Leu Ser Ser Leu Cys Ala Ala Ser Ala Glu Thr
          35                  40                  45

Glu Val Gly Ala Met Val Gly Ser Asn Val Val Leu Ser Cys Ile Asp
      50                  55                  60

Pro His Arg Arg His Phe Asn Leu Ser Gly Leu Tyr Val Tyr Trp Gln
 65                  70                  75                  80

Ile Glu Asn Pro Glu Val Ser Val Thr Tyr Tyr Leu Pro Tyr Lys Ser
              85                  90                  95

Pro Gly Ile Asn Val Asp Ser Ser Tyr Lys Asn Arg Gly His Leu Ser
              100                 105                 110

Leu Asp Ser Met Lys Gln Gly Asn Phe Ser Leu Tyr Leu Lys Asn Val
          115                 120                 125

Thr Pro Gln Asp Thr Gln Glu Phe Thr Cys Arg Val Phe Met Asn Thr
      130                 135                 140

Ala Thr Glu Leu Val Lys Ile Leu Glu Glu Val Val Arg Leu Arg Val
145                 150                 155                 160

Ala Ala Asn Phe Ser Thr Pro Val Ile Ser Thr Ser Asp Ser Ser Asn
              165                 170                 175

Pro Gly Gln Glu Arg Thr Tyr Thr Cys Met Ser Lys Asn Gly Tyr Pro
              180                 185                 190

Glu Pro Asn Leu Tyr Trp Ile Asn Thr Thr Asp Asn Ser Leu Ile Asp
          195                 200                 205

Thr Ala Leu Gln Asn Asn Thr Val Tyr Leu Asn Lys Leu Gly Leu Tyr
      210                 215                 220

Asp Val Ile Ser Thr Leu Arg Leu Pro Trp Thr Ser Arg Gly Asp Val
225                 230                 235                 240

Leu Cys Cys Val Glu Asn Val Ala Leu His Gln Asn Ile Thr Ser Ile
              245                 250                 255

Ser Gln Ala Glu Ser Phe Thr Gly Asn Asn Thr Lys Asn Pro Gln Glu
              260                 265                 270

Thr His Asn Asn Glu Leu Lys Val Leu Val Pro Val Leu Ala Val Leu
      275                 280                 285

Ala Ala Ala Ala Phe Val Ser Phe Ile Ile Tyr Arg Arg Thr Arg Pro
      290                 295                 300

His Arg Ser Tyr Thr Gly Pro Lys Thr Val Gln Leu Glu Leu Thr Asp
305                 310                 315                 320

His Ala
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<211> 306
<212> PRT
<213> mouse

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1               5                   10                  15

Pro Cys Pro Arg Leu Ile Leu Leu Phe Val Leu Leu Ile Arg Leu Ser
            20                  25                  30

Gln Val Ser Ser Asp Val Asp Glu Gln Leu Ser Lys Ser Val Lys Asp
        35                  40                  45

Lys Val Leu Leu Pro Cys Arg Tyr Asn Ser Pro His Glu Asp Glu Ser
    50                  55                  60

Glu Asp Arg Ile Tyr Trp Gln Lys His Asp Lys Val Val Leu Ser Val
65                  70                  75                  80

Ile Ala Gly Lys Leu Lys Val Trp Pro Glu Tyr Lys Asn Arg Thr Leu
                85                  90                  95

Tyr Asp Asn Thr Thr Tyr Ser Leu Ile Ile Leu Gly Leu Val Leu Ser
            100                 105                 110

Asp Arg Gly Thr Tyr Ser Cys Val Val Gln Lys Lys Glu Arg Gly Thr
        115                 120                 125

Tyr Glu Val Lys His Leu Ala Leu Val Lys Leu Ser Ile Lys Ala Asp
    130                 135                 140

Phe Ser Thr Pro Asn Ile Thr Glu Ser Gly Asn Pro Ser Ala Asp Thr
145                 150                 155                 160

Lys Arg Ile Thr Cys Phe Ala Ser Gly Gly Phe Pro Lys Pro Arg Phe
                165                 170                 175

Ser Trp Leu Glu Asn Gly Arg Glu Leu Pro Gly Ile Asn Thr Thr Ile
            180                 185                 190

Ser Gln Asp Pro Glu Ser Glu Leu Tyr Thr Ile Ser Ser Gln Leu Asp
        195                 200                 205

Phe Asn Thr Thr Arg Asn His Thr Ile Lys Cys Leu Ile Lys Tyr Gly
    210                 215                 220

Asp Ala His Val Ser Glu Asp Phe Thr Trp Glu Lys Pro Pro Glu Asp
225                 230                 235                 240

Pro Pro Asp Ser Lys Asn Thr Leu Val Leu Phe Gly Ala Gly Phe Gly
                245                 250                 255

Ala Val Ile Thr Val Val Val Ile Val Val Ile Ile Lys Cys Phe Cys
            260                 265                 270

Lys His Arg Ser Cys Phe Arg Arg Asn Glu Ala Ser Arg Glu Thr Asn
        275                 280                 285

Asn Ser Leu Thr Phe Gly Pro Glu Glu Ala Leu Ala Glu Gln Thr Val
    290                 295                 300

Phe Leu
305
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<223> Description of Artificial Sequence:Synthetic
      Oglionucleotide

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  1               5              10                  15

Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu Xaa
             20                  25                  30

Leu Phe Xaa Leu Leu Xaa Xaa Xaa Ser Xaa Xaa Xaa Xaa Xaa Xaa Xaa
         35                  40                  45

Xaa Xaa Xaa Xaa Xaa Xaa Val Xaa Xaa Xaa Val Xaa Leu Xaa Cys Xaa
         50                  55                  60

Xaa Xaa Xaa Xaa Xaa His Xaa Xaa Xaa Ser Xaa Xaa Xaa Xaa Tyr Trp
 65                  70                  75                  80

Gln Xaa Xaa Xaa Xaa Xaa Val Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa
                 85                  90                  95

Xaa Xaa Xaa Xaa Xaa Val Xaa Xaa Xaa Tyr Lys Asn Arg Xaa Xaa Xaa
             100                 105                 110

Xaa Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa Ser Leu Xaa Xaa Xaa Xaa
         115                 120                 125

Xaa Xaa Xaa Xaa Asp Xaa Xaa Xaa Xaa Xaa Cys Xaa Val Xaa Xaa Xaa
         130                 135                 140

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Val Xaa Leu Xaa
145                 150                 155                 160

Xaa Xaa Ala Xaa Phe Ser Thr Pro Xaa Ile Xaa Xaa Ser Xaa Xaa Xaa
             165                 170                 175

Xaa Xaa Xaa Xaa Xaa Arg Xaa Xaa Thr Cys Xaa Xaa Xaa Xaa Gly Xaa
             180                 185                 190

Pro Xaa Pro Xaa Xaa Xaa Trp Xaa Xaa Asn Xaa Xaa Xaa Xaa Xaa Xaa
         195                 200                 205

Ile Xaa Thr Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
         210                 215                 220

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Thr Xaa Xaa Xaa
225                 230                 235                 240

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Val Xaa Xaa Xaa Xaa Xaa Xaa Xaa
             245                 250                 255

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn Xaa Xaa Xaa Xaa Xaa
             260                 265                 270

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Val Xaa Val Xaa Val Xaa Xaa
```

```
                 275                      280                      285
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Phe Xaa Xaa Xaa Xaa Xaa
        290              295              300
Xaa Arg Xaa Xaa Xaa Xaa Ser Xaa Thr Xaa Gly Pro Xaa Xaa Xaa Xaa
305              310              315              320
Xaa Glu Xaa Thr Xaa Xaa Xaa
                325


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<212> DNA
<213> mouse

<220>
<221> CDS
<222> Complement((1)..(864))

<400> 11
atg cgg ctg ggc agt cct gga ctg ctc ttc ctg ctc ttc agc agc ctt      48
Met Arg Leu Gly Ser Pro Gly Leu Leu Phe Leu Leu Phe Ser Ser Leu
  1               5                  10                  15

cga gct gat act cag gag aag gaa gtc aga gcg atg gta ggc agc gac      96
Arg Ala Asp Thr Gln Glu Lys Glu Val Arg Ala Met Val Gly Ser Asp
                 20                  25                  30

gtg gag ctc agc tgc gct tgc cct gaa gga agc cgt ttt gat tta aat     144
Val Glu Leu Ser Cys Ala Cys Pro Glu Gly Ser Arg Phe Asp Leu Asn
             35                  40                  45

gat gtt tac gta tat tgg caa acc agt gag tcg aaa acc gtg gtg acc     192
Asp Val Tyr Val Tyr Trp Gln Thr Ser Glu Ser Lys Thr Val Val Thr
     50                  55                  60

tac cac atc cca cag aac agc tcc ttg gaa aac gtg gac agc cgc tac     240
Tyr His Ile Pro Gln Asn Ser Ser Leu Glu Asn Val Asp Ser Arg Tyr
 65                  70                  75                  80

cgg aac cga gcc ctg atg tca ccg gcc ggc atg ctg cgg ggc gac ttc     288
Arg Asn Arg Ala Leu Met Ser Pro Ala Gly Met Leu Arg Gly Asp Phe
                 85                  90                  95

tcc ctg cgc ttg ttc aac gtc acc ccc cag gac gag cag aag ttt cac     336
Ser Leu Arg Leu Phe Asn Val Thr Pro Gln Asp Glu Gln Lys Phe His
                100                 105                 110

tgc ctg gtg ttg agc caa tcc ctg gga ttc cag gag gtt ttg agc gtt     384
Cys Leu Val Leu Ser Gln Ser Leu Gly Phe Gln Glu Val Leu Ser Val
            115                 120                 125

gag gtt aca ctg cat gtg gca gca aac ttc agc gtg ccc gtc gtc agc     432
Glu Val Thr Leu His Val Ala Ala Asn Phe Ser Val Pro Val Val Ser
        130                 135                 140

gcc ccc cac agc ccc tcc cag gat gag ctc acc ttc acg tgt aca tcc     480
Ala Pro His Ser Pro Ser Gln Asp Glu Leu Thr Phe Thr Cys Thr Ser
145                 150                 155                 160

ata aac ggc tac ccc agg ccc aac gtg tac tgg atc aat aag acg gac     528
Ile Asn Gly Tyr Pro Arg Pro Asn Val Tyr Trp Ile Asn Lys Thr Asp
```

```
                    165                      170                        175
aac agc ctg ctg gac cag gct ctg cag aat gac acc gtc ttc ttg aac       576
Asn Ser Leu Leu Asp Gln Ala Leu Gln Asn Asp Thr Val Phe Leu Asn
            180                 185                 190

atg cgg ggc ttg tat gac gtg gtc agc gtg ctg agg atc gca cgg acc       624
Met Arg Gly Leu Tyr Asp Val Val Ser Val Leu Arg Ile Ala Arg Thr
            195                 200                 205

ccc agc gtg aac att ggc tgc tgc ata gag aac gtg ctt ctg cag cag       672
Pro Ser Val Asn Ile Gly Cys Cys Ile Glu Asn Val Leu Leu Gln Gln
        210                 215                 220

aac ctg act gtc ggc agc cag aca gga aat gac atc gga gag aga gac       720
Asn Leu Thr Val Gly Ser Gln Thr Gly Asn Asp Ile Gly Glu Arg Asp
225                 230                 235                 240

aag atc aca gag aat cca gtc agt acc ggc gag aaa aac gcg gcc acg       768
Lys Ile Thr Glu Asn Pro Val Ser Thr Gly Glu Lys Asn Ala Ala Thr
                245                 250                 255

tgg agc atc ctg gct gtc ctg tgc ctg ctt gtg gtc gtg gcg gtg gcc       816
Trp Ser Ile Leu Ala Val Leu Cys Leu Leu Val Val Val Ala Val Ala
            260                 265                 270

ata ggc tgg gtg tgc agg gac cga tgc ctc caa cac agc tat gca ggt       864
Ile Gly Trp Val Cys Arg Asp Arg Cys Leu Gln His Ser Tyr Ala Gly
            275                 280                 285


<210> 12
<211> 288
<212> PRT
<213> mouse

<400> 12
Met Arg Leu Gly Ser Pro Gly Leu Leu Phe Leu Leu Phe Ser Ser Leu
  1               5                  10                  15

Arg Ala Asp Thr Gln Glu Lys Glu Val Arg Ala Met Val Gly Ser Asp
            20                  25                  30

Val Glu Leu Ser Cys Ala Cys Pro Glu Gly Ser Arg Phe Asp Leu Asn
        35                  40                  45

Asp Val Tyr Val Tyr Trp Gln Thr Ser Glu Ser Lys Thr Val Val Thr
    50                  55                  60

Tyr His Ile Pro Gln Asn Ser Ser Leu Glu Asn Val Asp Ser Arg Tyr
65                  70                  75                  80

Arg Asn Arg Ala Leu Met Ser Pro Ala Gly Met Leu Arg Gly Asp Phe
                85                  90                  95

Ser Leu Arg Leu Phe Asn Val Thr Pro Gln Asp Glu Gln Lys Phe His
            100                 105                 110

Cys Leu Val Leu Ser Gln Ser Leu Gly Phe Gln Glu Val Leu Ser Val
            115                 120                 125

Glu Val Thr Leu His Val Ala Ala Asn Phe Ser Val Pro Val Val Ser
    130                 135                 140
```

```
Ala Pro His Ser Pro Ser Gln Asp Glu Leu Thr Phe Thr Cys Thr Ser
145             150             155             160

Ile Asn Gly Tyr Pro Arg Pro Asn Val Tyr Trp Ile Asn Lys Thr Asp
            165             170             175

Asn Ser Leu Leu Asp Gln Ala Leu Gln Asn Asp Thr Val Phe Leu Asn
        180             185             190

Met Arg Gly Leu Tyr Asp Val Val Ser Val Leu Arg Ile Ala Arg Thr
        195             200             205

Pro Ser Val Asn Ile Gly Cys Cys Ile Glu Asn Val Leu Leu Gln Gln
    210             215             220

Asn Leu Thr Val Gly Ser Gln Thr Gly Asn Asp Ile Gly Glu Arg Asp
225             230             235             240

Lys Ile Thr Glu Asn Pro Val Ser Thr Gly Glu Lys Asn Ala Ala Thr
            245             250             255

Trp Ser Ile Leu Ala Val Leu Cys Leu Leu Val Val Val Ala Val Ala
            260             265             270

Ile Gly Trp Val Cys Arg Asp Arg Cys Leu Gln His Ser Tyr Ala Gly
        275             280             285


<210> 13
<211> 267
<212> PRT
<213> Human

<400> 13
Glu Lys Glu Val Arg Ala Met Val Gly Ser Asp Val Glu Leu Ser Cys
  1           5           10              15

Ala Cys Pro Glu Gly Ser Arg Phe Asp Leu Asn Asp Val Tyr Val Tyr
            20              25              30

Trp Gln Thr Ser Glu Ser Lys Thr Val Val Thr Tyr His Ile Pro Gln
        35              40              45

Asn Ser Ser Leu Glu Asn Val Asp Ser Arg Tyr Arg Asn Arg Ala Leu
    50              55              60

Met Ser Pro Ala Gly Met Leu Arg Gly Asp Phe Ser Leu Arg Leu Phe
65              70              75              80

Asn Val Thr Pro Gln Asp Glu Gln Lys Phe His Cys Leu Val Leu Ser
            85              90              95

Gln Ser Leu Gly Phe Gln Glu Val Leu Ser Val Glu Val Thr Leu His
        100             105             110

Val Ala Ala Asn Phe Ser Val Pro Val Val Ser Ala Pro His Ser Pro
        115             120             125

Ser Gln Asp Glu Leu Thr Phe Thr Cys Thr Ser Ile Asn Gly Tyr Pro
    130             135             140

Arg Pro Asn Val Tyr Trp Ile Asn Lys Thr Asp Asn Ser Leu Leu Asp
145             150             155             160
```

```
Gln Ala Leu Gln Asn Asp Thr Val Phe Leu Asn Met Arg Gly Leu Tyr
                165             170                 175

Asp Val Val Ser Val Leu Arg Ile Ala Arg Thr Pro Ser Val Asn Ile
            180             185             190

Gly Cys Cys Ile Glu Asn Val Leu Leu Gln Gln Asn Leu Thr Val Gly
        195             200             205

Ser Gln Thr Gly Asn Asp Ile Gly Glu Arg Asp Lys Ile Thr Glu Asn
    210             215             220

Pro Val Ser Thr Gly Glu Lys Asn Ala Ala Thr Trp Ser Ile Leu Ala
225             230             235             240

Val Leu Cys Leu Leu Val Val Val Ala Val Ala Ile Gly Trp Val Cys
            245             250             255

Arg Asp Arg Cys Leu Gln His Ser Tyr Ala Gly
            260             265
```

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<210> 14
<211> 276
<212> PRT
<213> mouse

<400> 14
Glu Thr Glu Val Gly Ala Met Val Gly Ser Asn Val Val Leu Ser Cys
  1               5                 10                  15

Ile Asp Pro His Arg Arg His Phe Asn Leu Ser Gly Leu Tyr Val Tyr
            20              25                  30

Trp Gln Ile Glu Asn Pro Glu Val Ser Val Thr Tyr Tyr Leu Pro Tyr
        35              40                  45

Lys Ser Pro Gly Ile Asn Val Asp Ser Ser Tyr Lys Asn Arg Gly His
    50              55                  60

Leu Ser Leu Asp Ser Met Lys Gln Gly Asn Phe Ser Leu Tyr Leu Lys
65              70                  75                  80

Asn Val Thr Pro Gln Asp Thr Gln Glu Phe Thr Cys Arg Val Phe Met
            85                  90                  95

Asn Thr Ala Thr Glu Leu Val Lys Ile Leu Glu Glu Val Val Arg Leu
            100             105             110

Arg Val Ala Ala Asn Phe Ser Thr Pro Val Ile Ser Thr Ser Asp Ser
        115             120             125

Ser Asn Pro Gly Gln Glu Arg Thr Tyr Thr Cys Met Ser Lys Asn Gly
    130             135             140

Tyr Pro Glu Pro Asn Leu Tyr Trp Ile Asn Thr Thr Asp Asn Ser Leu
145             150             155             160

Ile Asp Thr Ala Leu Gln Asn Asn Thr Val Tyr Leu Asn Lys Leu Gly
            165             170             175

Leu Tyr Asp Val Ile Ser Thr Leu Arg Leu Pro Trp Thr Ser Arg Gly
            180             185             190
```

```
Asp Val Leu Cys Cys Val Glu Asn Val Ala Leu His Gln Asn Ile Thr
        195                 200             205

Ser Ile Ser Gln Ala Glu Ser Phe Thr Gly Asn Asn Thr Lys Asn Pro
    210                 215             220

Gln Glu Thr His Asn Asn Glu Leu Lys Val Leu Val Pro Val Leu Ala
225                 230             235                 240

Val Leu Ala Ala Ala Ala Phe Val Ser Phe Ile Ile Tyr Arg Arg Thr
            245                 250                 255

Arg Pro His Arg Ser Tyr Thr Gly Pro Lys Thr Val Gln Leu Glu Leu
            260             265             270

Thr Asp His Ala
        275


<210> 15
<211> 280
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Synthetic
      Oglionucleotide

<400> 15
Glu Xaa Glu Val Xaa Ala Met Gly Val Ser Xaa Val Xaa Leu Ser Cys
  1             5               10              15

Xaa Xaa Pro Xaa Xaa Xaa Xaa Phe Xaa Leu Xaa Xaa Xaa Tyr Val Tyr
        20              25              30

Trp Gln Xaa Xaa Xaa Xaa Xaa Xaa Val Thr Tyr Xaa Xaa Pro Xaa
        35              40              45

Xaa Ser Xaa Xaa Xaa Asn Val Asp Ser Xaa Tyr Xaa Asn Arg Xaa Xaa
    50              55              60

Xaa Ser Xaa Xaa Xaa Met Xaa Xaa Gly Xaa Phe Ser Leu Xaa Leu Xaa
65              70              75              80

Asn Val Thr Pro Gln Asp Xaa Gln Xaa Phe Xaa Cys Xaa Val Xaa Xaa
            85              90                  95

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa Val Xaa Leu
        100             105             110

Xaa Val Ala Ala Asn Phe Ser Xaa Pro Val Xaa Ser Xaa Xaa Xaa Ser
    115             120             125

Xaa Xaa Xaa Xaa Xaa Glu Xaa Thr Xaa Thr Cys Xaa Ser Xaa Asn Gly
    130             135             140

Tyr Pro Xaa Pro Asn Xaa Tyr Trp Ile Asn Xaa Thr Asp Asn Ser Leu
145             150             155             160

Xaa Asp Xaa Ala Leu Gln Asn Xaa Thr Val Xaa Leu Asn Xaa Xaa Gly
        165             170             175

Leu Tyr Asp Val Xaa Ser Xaa Leu Arg Xaa Xaa Xaa Thr Xaa Xaa Xaa
```

```
                    180                    185                    190
Xaa Xaa Xaa Cys Cys Xaa Glu Asn Val Xaa Leu Xaa Gln Asn Xaa Thr
        195                200                205

Xaa Xaa Ser Gln Xaa Xaa Xaa Xaa Xaa Gly Xaa Xaa Lys Xaa Xaa Xaa
        210                215                220

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Lys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu
225                230                235                        240

Ala Val Leu Xaa Xaa Xaa Xaa Xaa Val Xaa Xaa Xaa Ile Xaa Xaa Xaa
            245                250                255

Xaa Arg Xaa Arg Xaa Xaa Xaa Xaa Ser Tyr Xaa Gly Xaa Xaa Xaa Xaa
            260                265                270

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        275                280


<210> 16
<211> 1294
<212> DNA
<213> Human

<220>
<221> 5'UTR
<222> (1)..(199)

<220>
<221> CDS
<222> (200)..(1105)

<400> 16
gctggtacgc ctgcaggtac cggtccggaa ttcccgggtc gacccacgcg tccgcccacg 60

cgtccgcggg agcgcagtta gagccgatct cccgcgcccc gaggttgctc ctctccgagg 120

tctcccgcgg cccaagttct ccgcgccccg aggtctccgc gccccgaggt ctccgcggcc 180

cgaggtctcc gcccgcacc atg cgg ctg ggc agt cct gga ctg ctc ttc ctg 232
                      Met Arg Leu Gly Ser Pro Gly Leu Leu Phe Leu
                       1               5                   10

ctc ttc agc agc ctt cga gct gat act cag gag aag gaa gtc aga gcg    280
Leu Phe Ser Ser Leu Arg Ala Asp Thr Gln Glu Lys Glu Val Arg Ala
            15                  20                  25

atg gta ggc agc gac gtg gag ctc agc tgc gct tgc cct gaa gga agc    328
Met Val Gly Ser Asp Val Glu Leu Ser Cys Ala Cys Pro Glu Gly Ser
        30                  35                  40

cgt ttt gat tta aat gat gtt tac gta tat tgg caa acc agt gag tcg    376
Arg Phe Asp Leu Asn Asp Val Tyr Val Tyr Trp Gln Thr Ser Glu Ser
        45                  50                  55

aaa acc gtg gtg acc tac cac atc cca cag aac agc tcc ttg gaa aac    424
Lys Thr Val Val Thr Tyr His Ile Pro Gln Asn Ser Ser Leu Glu Asn
60                  65                  70                  75

gtg gac agc cgc tac cgg aac cga gcc ctg atg tca ccg gcc ggc atg    472
Val Asp Ser Arg Tyr Arg Asn Arg Ala Leu Met Ser Pro Ala Gly Met
                80                  85                  90
```

```
ctg cgg ggc gac ttc tcc ctg cgc ttg ttc aac gtc acc ccc cag gac    520
Leu Arg Gly Asp Phe Ser Leu Arg Leu Phe Asn Val Thr Pro Gln Asp
             95                  100                 105

gag cag aag ttt cac tgc ctg gtg ttg agc caa tcc ctg gga ttc cag    568
Glu Gln Lys Phe His Cys Leu Val Leu Ser Gln Ser Leu Gly Phe Gln
         110                 115                 120

gag gtt ttg agc gtt gag gtt aca ctg cat gtg gca gca aac ttc agc    616
Glu Val Leu Ser Val Glu Val Thr Leu His Val Ala Ala Asn Phe Ser
         125                 130                 135

gtg ccc gtc gtc agc gcc ccc cac agc ccc tcc cag gat gag ctc acc    664
Val Pro Val Val Ser Ala Pro His Ser Pro Ser Gln Asp Glu Leu Thr
140                 145                 150                 155

ttc acg tgt aca tcc ata aac ggc tac ccc agg ccc aac gtg tac tgg    712
Phe Thr Cys Thr Ser Ile Asn Gly Tyr Pro Arg Pro Asn Val Tyr Trp
                 160                 165                 170

atc aat aag acg gac aac agc ctg ctg gac cag gct ctg cag aat gac    760
Ile Asn Lys Thr Asp Asn Ser Leu Leu Asp Gln Ala Leu Gln Asn Asp
             175                 180                 185

acc gtc ttc ttg aac atg cgg ggc ttg tat gac gtg gtc agc gtg ctg    808
Thr Val Phe Leu Asn Met Arg Gly Leu Tyr Asp Val Val Ser Val Leu
             190                 195                 200

agg atc gca cgg acc ccc agc gtg aac att ggc tgc tgc ata gag aac    856
Arg Ile Ala Arg Thr Pro Ser Val Asn Ile Gly Cys Cys Ile Glu Asn
205                 210                 215

gtg ctt ctg cag cag aac ctg act gtc ggc agc cag aca gga aat gac    904
Val Leu Leu Gln Gln Asn Leu Thr Val Gly Ser Gln Thr Gly Asn Asp
220                 225                 230                 235

atc gga gag aga gac aag atc aca gag aat cca gtc agt acc ggc gag    952
Ile Gly Glu Arg Asp Lys Ile Thr Glu Asn Pro Val Ser Thr Gly Glu
                 240                 245                 250

aaa aac gcg gcc acg tgg agc atc ctg gct gtc ctg tgc ctg ctt gtg    1000
Lys Asn Ala Ala Thr Trp Ser Ile Leu Ala Val Leu Cys Leu Leu Val
             255                 260                 265

gtc gtg gcg gtg gcc ata ggc tgg gtg tgc agg gac cga tgc ctc caa    1048
Val Val Ala Val Ala Ile Gly Trp Val Cys Arg Asp Arg Cys Leu Gln
             270                 275                 280

cac agc tat gca ggt gcc tgg gct gtg agt ccg gag aca gag ctc act    1096
His Ser Tyr Ala Gly Ala Trp Ala Val Ser Pro Glu Thr Glu Leu Thr
             285                 290                 295

ggc cac gtt tgaccggagc tcaccgccca gagcgtggac agggcttccg            1145
Gly His Val
300

tgagacgcca ccgtgagagg ccaggtggca gcttgagcat ggactcccag actgcagggg 1205

agcacttggg gcagccccca gaaggaccac tgctggatcc cagggagaac ctgctggcgt 1265

tggctgtgat cctggaatga ggccctttc                                    1294
```

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<210> 17
<211> 302
<212> PRT
<213> Human

<400> 17
Met Arg Leu Gly Ser Pro Gly Leu Leu Phe Leu Leu Phe Ser Ser Leu
 1           5               10              15

Arg Ala Asp Thr Gln Glu Lys Glu Val Arg Ala Met Val Gly Ser Asp
             20              25              30

Val Glu Leu Ser Cys Ala Cys Pro Glu Gly Ser Arg Phe Asp Leu Asn
         35              40              45

Asp Val Tyr Val Tyr Trp Gln Thr Ser Glu Ser Lys Thr Val Val Thr
     50              55              60

Tyr His Ile Pro Gln Asn Ser Ser Leu Glu Asn Val Asp Ser Arg Tyr
65              70              75              80

Arg Asn Arg Ala Leu Met Ser Pro Ala Gly Met Leu Arg Gly Asp Phe
             85              90              95

Ser Leu Arg Leu Phe Asn Val Thr Pro Gln Asp Glu Gln Lys Phe His
         100             105             110

Cys Leu Val Leu Ser Gln Ser Leu Gly Phe Gln Glu Val Leu Ser Val
     115             120             125

Glu Val Thr Leu His Val Ala Ala Asn Phe Ser Val Pro Val Val Ser
    130             135             140

Ala Pro His Ser Pro Ser Gln Asp Glu Leu Thr Phe Thr Cys Thr Ser
145             150             155             160

Ile Asn Gly Tyr Pro Arg Pro Asn Val Tyr Trp Ile Asn Lys Thr Asp
             165             170             175

Asn Ser Leu Leu Asp Gln Ala Leu Gln Asn Asp Thr Val Phe Leu Asn
         180             185             190

Met Arg Gly Leu Tyr Asp Val Val Ser Val Leu Arg Ile Ala Arg Thr
         195             200             205

Pro Ser Val Asn Ile Gly Cys Cys Ile Glu Asn Val Leu Leu Gln Gln
    210             215             220

Asn Leu Thr Val Gly Ser Gln Thr Gly Asn Asp Ile Gly Glu Arg Asp
225             230             235             240

Lys Ile Thr Glu Asn Pro Val Ser Thr Gly Glu Lys Asn Ala Ala Thr
             245             250             255

Trp Ser Ile Leu Ala Val Leu Cys Leu Leu Val Val Val Ala Val Ala
             260             265             270

Ile Gly Trp Val Cys Arg Asp Arg Cys Leu Gln His Ser Tyr Ala Gly
             275             280             285

Ala Trp Ala Val Ser Pro Glu Thr Glu Leu Thr Gly His Val
    290             295             300
```

```
<210> 18
<211> 302
<212> PRT
<213> Human

<400> 18
Met Arg Leu Gly Ser Pro Gly Leu Leu Phe Leu Leu Phe Ser Ser Leu
  1               5                  10                  15

Arg Ala Asp Thr Gln Glu Lys Glu Val Arg Ala Met Val Gly Ser Asp
               20                  25                  30

Val Glu Leu Ser Cys Ala Cys Pro Glu Gly Ser Arg Phe Asp Leu Asn
           35                  40                  45

Asp Val Tyr Val Tyr Trp Gln Thr Ser Glu Ser Lys Thr Val Val Thr
       50                  55                  60

Tyr His Ile Pro Gln Asn Ser Ser Leu Glu Asn Val Asp Ser Arg Tyr
 65                  70                  75                  80

Arg Asn Arg Ala Leu Met Ser Pro Ala Gly Met Leu Arg Gly Asp Phe
               85                  90                  95

Ser Leu Arg Leu Phe Asn Val Thr Pro Gln Asp Glu Gln Lys Phe His
              100                 105                 110

Cys Leu Val Leu Ser Gln Ser Leu Gly Phe Gln Glu Val Leu Ser Val
              115                 120                 125

Glu Val Thr Leu His Val Ala Ala Asn Phe Ser Val Pro Val Val Ser
          130                 135                 140

Ala Pro His Ser Pro Ser Gln Asp Glu Leu Thr Phe Thr Cys Thr Ser
145                 150                 155                 160

Ile Asn Gly Tyr Pro Arg Pro Asn Val Tyr Trp Ile Asn Lys Thr Asp
                165                 170                 175

Asn Ser Leu Leu Asp Gln Ala Leu Gln Asn Asp Thr Val Phe Leu Asn
              180                 185                 190

Met Arg Gly Leu Tyr Asp Val Val Ser Val Leu Arg Ile Ala Arg Thr
          195                 200                 205

Pro Ser Val Asn Ile Gly Cys Cys Ile Glu Asn Val Leu Leu Gln Gln
    210                 215                 220

Asn Leu Thr Val Gly Ser Gln Thr Gly Asn Asp Ile Gly Glu Arg Asp
225                 230                 235                 240

Lys Ile Thr Glu Asn Pro Val Ser Thr Gly Glu Lys Asn Ala Ala Thr
                245                 250                 255

Trp Ser Ile Leu Ala Val Leu Cys Leu Leu Val Val Val Ala Val Ala
          260                 265                 270

Ile Gly Trp Val Cys Arg Asp Arg Cys Leu Gln His Ser Tyr Ala Gly
          275                 280                 285

Ala Trp Ala Val Ser Pro Glu Thr Glu Leu Thr Gly His Val
          290                 295                 300
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```
<210> 19
<211> 322
<212> PRT
<213> mouse

<400> 19
Met Gln Leu Lys Cys Pro Cys Phe Val Ser Leu Gly Thr Arg Gln Pro
 1               5                  10                  15

Val Trp Lys Lys Leu His Val Ser Ser Gly Phe Phe Ser Gly Leu Gly
                20                  25                  30

Leu Phe Leu Leu Leu Leu Ser Ser Leu Cys Ala Ala Ser Ala Glu Thr
            35                  40                  45

Glu Val Gly Ala Met Val Gly Ser Asn Val Val Leu Ser Cys Ile Asp
    50                  55                  60

Pro His Arg Arg His Phe Asn Leu Ser Gly Leu Tyr Val Tyr Trp Gln
65                  70                  75                  80

Ile Glu Asn Pro Glu Val Ser Val Thr Tyr Tyr Leu Pro Tyr Lys Ser
                85                  90                  95

Pro Gly Ile Asn Val Asp Ser Ser Tyr Lys Asn Arg Gly His Leu Ser
            100                 105                 110

Leu Asp Ser Met Lys Gln Gly Asn Phe Ser Leu Tyr Leu Lys Asn Val
        115                 120                 125

Thr Pro Gln Asp Thr Gln Glu Phe Thr Cys Arg Val Phe Met Asn Thr
    130                 135                 140

Ala Thr Glu Leu Val Lys Ile Leu Glu Glu Val Val Arg Leu Arg Val
145                 150                 155                 160

Ala Ala Asn Phe Ser Thr Pro Val Ile Ser Thr Ser Asp Ser Ser Asn
                165                 170                 175

Pro Gly Gln Glu Arg Thr Tyr Thr Cys Met Ser Lys Asn Gly Tyr Pro
            180                 185                 190

Glu Pro Asn Leu Tyr Trp Ile Asn Thr Thr Asp Asn Ser Leu Ile Asp
        195                 200                 205

Thr Ala Leu Gln Asn Asn Thr Val Tyr Leu Asn Lys Leu Gly Leu Tyr
    210                 215                 220

Asp Val Ile Ser Thr Leu Arg Leu Pro Trp Thr Ser Arg Gly Asp Val
225                 230                 235                 240

Leu Cys Cys Val Glu Asn Val Ala Leu His Gln Asn Ile Thr Ser Ile
                245                 250                 255

Ser Gln Ala Glu Ser Phe Thr Gly Asn Asn Thr Lys Asn Pro Gln Glu
            260                 265                 270

Thr His Asn Asn Glu Leu Lys Val Leu Val Pro Val Leu Ala Val Leu
        275                 280                 285

Ala Ala Ala Ala Phe Val Ser Phe Ile Ile Tyr Arg Arg Thr Arg Pro
    290                 295                 300
```

```
His Arg Ser Tyr Thr Gly Pro Lys Thr Val Gln Leu Glu Leu Thr Asp
305                 310             315                 320

His Ala


<210> 20
<211> 329
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Synthetic
      Oglionucleotide

<400> 20
Met Xaa Leu Xaa Xaa Pro Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
  1               5                 10                  15

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Gly Leu Xaa
            20                  25                  30

Leu Phe Xaa Leu Leu Xaa Ser Ser Leu Xaa Ala Xaa Xaa Xaa Glu Xaa
        35                  40                  45

Glu Val Xaa Ala Met Val Gly Ser Xaa Val Xaa Leu Ser Cys Xaa Xaa
    50                  55                  60

Pro Xaa Xaa Xaa Xaa Phe Xaa Leu Xaa Xaa Xaa Tyr Val Tyr Trp Gln
65                  70                  75                  80

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Val Thr Tyr Xaa Xaa Pro Xaa Xaa Ser
                85                  90                  95

Xaa Xaa Xaa Asn Val Asp Ser Xaa Tyr Xaa Asn Arg Xaa Xaa Xaa Ser
            100                 105                 110

Xaa Xaa Xaa Met Xaa Xaa Gly Xaa Phe Ser Leu Xaa Leu Xaa Asn Val
        115                 120                 125

Thr Pro Gln Asp Xaa Gln Xaa Phe Xaa Cys Xaa Val Xaa Xaa Xaa Xaa
    130             135             140

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa Val Xaa Leu Xaa Val
145                 150                 155                 160

Ala Ala Asn Phe Ser Xaa Pro Val Xaa Ser Xaa Xaa Xaa Ser Xaa Xaa
            165                 170                 175

Xaa Xaa Xaa Glu Xaa Thr Xaa Thr Cys Xaa Ser Xaa Asn Gly Tyr Pro
        180             185             190

Xaa Pro Asn Xaa Tyr Trp Ile Asn Xaa Thr Asp Asn Ser Leu Xaa Asp
    195             200             205

Xaa Ala Leu Gln Asn Xaa Thr Val Xaa Leu Asn Xaa Xaa Gly Leu Tyr
    210             215             220

Asp Val Xaa Ser Xaa Leu Arg Xaa Xaa Xaa Thr Xaa Xaa Xaa Xaa Xaa
225             230             235                 240

Xaa Cys Cys Xaa Glu Asn Val Xaa Leu Xaa Gln Asn Xaa Thr Xaa Xaa
        245             250             255
```

```
Ser Gln Xaa Xaa Xaa Xaa Xaa Gly Xaa Xaa Xaa Lys Xaa Xaa Xaa Xaa
        260             265             270
Xaa Xaa Xaa Xaa Xaa Xaa Lys Xaa Xaa Xaa Xaa Xaa Xaa Leu Ala
        275             280             285
Val Leu Xaa Xaa Xaa Xaa Xaa Val Xaa Xaa Xaa Ile Xaa Xaa Xaa Xaa
    290             295             300
Arg Xaa Arg Xaa Xaa Xaa Xaa Ser Tyr Xaa Gly Xaa Xaa Xaa Val Xaa
305             310             315             320
Xaa Glu Xaa Xaa Leu Thr Xaa His Xaa
            325
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<210> 21
<211> 1370
<212> DNA
<213> Human

<220>
<221> 5'UTR
<222> (1)..(165)

<220>
<221> CDS
<222> (166)..(762)

<400> 21
aacaatttca cacaggaaac agctatgacc atgattacgc caagctctaa tacgactcac 60

tatagggaaa gctggtacgc ctgcaggtac cggtccggaa ttcccgggtc gacccacgcg 120

tccgtgaaca ctgaacgcga ggactgttaa ctgtttctgg caaac atg aag tca ggc 177
                                                  Met Lys Ser Gly
                                                   1

ctc tgg tat ttc ttt ctc ttc tgc ttg cgc att aaa gtt tta aca gga 225
Leu Trp Tyr Phe Phe Leu Phe Cys Leu Arg Ile Lys Val Leu Thr Gly
 5               10              15              20

gaa atc aat ggt tct gcc aat tat gag atg ttt ata ttt cac aac gga 273
Glu Ile Asn Gly Ser Ala Asn Tyr Glu Met Phe Ile Phe His Asn Gly
                25              30              35

ggt gta caa att tta tgc aaa tat cct gac att gtc cag caa ttt aaa 321
Gly Val Gln Ile Leu Cys Lys Tyr Pro Asp Ile Val Gln Gln Phe Lys
            40              45              50

atg cag ttg ctg aaa ggg ggg caa ata ctc tgc gat ctc act aag aca 369
Met Gln Leu Leu Lys Gly Gly Gln Ile Leu Cys Asp Leu Thr Lys Thr
        55              60              65

aaa gga agt gga aac aca gtg tcc att aag agt ctg aaa ttc tgc cat 417
Lys Gly Ser Gly Asn Thr Val Ser Ile Lys Ser Leu Lys Phe Cys His
    70              75              80

tct cag tta tcc aac aac agt gtc tct ttt ttt cta tac aac ttg gac 465
Ser Gln Leu Ser Asn Asn Ser Val Ser Phe Phe Leu Tyr Asn Leu Asp
85              90              95              100

cat tct cat gcc aac tat tac ttc tgc aac cta tca att ttt gat cct 513
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His Ser His Ala Asn Tyr Tyr Phe Cys Asn Leu Ser Ile Phe Asp Pro
            105             110                 115

cct cct ttt aaa gta act ctt aca gga gga tat ttg cat att tat gaa    561
Pro Pro Phe Lys Val Thr Leu Thr Gly Gly Tyr Leu His Ile Tyr Glu
            120             125                 130

tca caa ctt tgt tgc cag ctg aag ttc tgg tta ccc ata gga tgt gca    609
Ser Gln Leu Cys Cys Gln Leu Lys Phe Trp Leu Pro Ile Gly Cys Ala
            135             140                 145

gcc ttt gtt gta gtc tgc att ttg gga tgc ata ctt att tgt tgg ctt    657
Ala Phe Val Val Val Cys Ile Leu Gly Cys Ile Leu Ile Cys Trp Leu
            150             155                 160

aca aaa aag aag tat tca tcc agt gtg cac gac cct aac ggt gaa tac    705
Thr Lys Lys Lys Tyr Ser Ser Ser Val His Asp Pro Asn Gly Glu Tyr
165             170                 175                 180

atg ttc atg aga gca gtg aac aca gcc aaa aaa tct aga ctc aca gat    753
Met Phe Met Arg Ala Val Asn Thr Ala Lys Lys Ser Arg Leu Thr Asp
                185             190                 195

gtg acc cta taatatggaa ctctggcacc caggcatgaa gcacgttggc            802
Val Thr Leu

cagttttcct caacttgaag tgcaagattc tcttatttcc gggaccacgg agagtctgac  862

ttaactacat acatcttctg ctggtgtttt gttcaatctg gaagaatgac tgtatcagtc  922

aatggggatt ttaacagact gccttggtac tgccgagtcc tctcaaaaca aacaccctct  982

tgcaaccagc tttggagaaa gcccagctcc tgtgtgctca ctgggagtgg aatccctgtc  1042

tccacatctg ctcctagcag tgcatcagcc agtaaaacaa acacatttac aagaaaaatg  1102

ttttaaagat gccaggggta ctgaatctgc aaagcaaatg agcagccaag gaccagcatc  1162

tgtccgcatt tcactatcat actacctctt ctttctgtag ggatgagaat tcctcttttta 1222

atcagtcaag ggagatgctt caaagctgga gctattttat ttctgagatg ttgatgtgaa  1282

ctgtacatta gtacatactc agtactctcc ttcaattgct gaaccccagt tgaccatttt  1342

accaagactt tagatgcttt cttgtgcc                                    1370
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<210> 22
<211> 199
<212> PRT
<213> Human

<400> 22
Met Lys Ser Gly Leu Trp Tyr Phe Phe Leu Phe Cys Leu Arg Ile Lys
1               5                   10                  15

Val Leu Thr Gly Glu Ile Asn Gly Ser Ala Asn Tyr Glu Met Phe Ile
            20                  25                  30

Phe His Asn Gly Gly Val Gln Ile Leu Cys Lys Tyr Pro Asp Ile Val
            35                  40                  45

Gln Gln Phe Lys Met Gln Leu Leu Lys Gly Gly Gln Ile Leu Cys Asp
    50                  55                  60
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Leu Thr Lys Thr Lys Gly Ser Gly Asn Thr Val Ser Ile Lys Ser Leu
65                  70                  75                  80

Lys Phe Cys His Ser Gln Leu Ser Asn Asn Ser Val Ser Phe Phe Leu
                85                  90                  95

Tyr Asn Leu Asp His Ser His Ala Asn Tyr Tyr Phe Cys Asn Leu Ser
            100                 105                 110

Ile Phe Asp Pro Pro Pro Phe Lys Val Thr Leu Thr Gly Gly Tyr Leu
            115                 120                 125

His Ile Tyr Glu Ser Gln Leu Cys Cys Gln Leu Lys Phe Trp Leu Pro
    130                 135                 140

Ile Gly Cys Ala Ala Phe Val Val Val Cys Ile Leu Gly Cys Ile Leu
145                 150                 155                 160

Ile Cys Trp Leu Thr Lys Lys Lys Tyr Ser Ser Ser Val His Asp Pro
                165                 170                 175

Asn Gly Glu Tyr Met Phe Met Arg Ala Val Asn Thr Ala Lys Lys Ser
            180                 185                 190

Arg Leu Thr Asp Val Thr Leu
            195
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<210> 23
<211> 199
<212> PRT
<213> Human

<400> 23
Met Lys Ser Gly Leu Trp Tyr Phe Phe Leu Phe Cys Leu Arg Ile Lys

1                   5                   10                  15

Val Leu Thr Gly Glu Ile Asn Gly Ser Ala Asn Tyr Glu Met Phe Ile
            20                  25                  30

Phe His Asn Gly Gly Val Gln Ile Leu Cys Lys Tyr Pro Asp Ile Val
        35                  40                  45

Gln Gln Phe Lys Met Gln Leu Leu Lys Gly Gly Gln Ile Leu Cys Asp
    50                  55                  60

Leu Thr Lys Thr Lys Gly Ser Gly Asn Thr Val Ser Ile Lys Ser Leu
65                  70                  75                  80

Lys Phe Cys His Ser Gln Leu Ser Asn Asn Ser Val Ser Phe Phe Leu
                85                  90                  95

Tyr Asn Leu Asp His Ser His Ala Asn Tyr Tyr Phe Cys Asn Leu Ser
            100                 105                 110

Ile Phe Asp Pro Pro Pro Phe Lys Val Thr Leu Thr Gly Gly Tyr Leu
            115                 120                 125

His Ile Tyr Glu Ser Gln Leu Cys Cys Gln Leu Lys Phe Trp Leu Pro
    130                 135                 140
```

Ile Gly Cys Ala Ala Phe Val Val Val Cys Ile Leu Gly Cys Ile Leu
145              150          155              160

Ile Cys Trp Leu Thr Lys Lys Lys Tyr Ser Ser Ser Val His Asp Pro
            165              170              175

Asn Gly Glu Tyr Met Phe Met Arg Ala Val Asn Thr Ala Lys Lys Ser
            180              185              190

Arg Leu Thr Asp Val Thr Leu
        195


<210> 24
<211> 200
<212> PRT
<213> mouse

<400> 24
Met Lys Pro Tyr Phe Cys Arg Val Phe Val Phe Cys Phe Leu Ile Arg
  1           5              10              15

Leu Leu Thr Gly Glu Ile Asn Gly Ser Ala Asp His Arg Met Phe Ser
            20              25              30

Phe His Asn Gly Gly Val Gln Ile Ser Cys Lys Tyr Pro Glu Thr Val
        35              40              45

Gln Gln Leu Lys Met Arg Leu Phe Arg Glu Arg Glu Val Leu Cys Glu
    50              55              60

Leu Thr Lys Thr Lys Gly Ser Gly Asn Ala Val Ser Ile Lys Asn Pro
65              70              75              80

Met Leu Cys Leu Tyr His Leu Ser Asn Asn Ser Val Ser Phe Phe Leu
            85              90              95

Asn Asn Pro Asp Ser Ser Gln Gly Ser Tyr Tyr Phe Cys Ser Leu Ser
            100             105             110

Ile Phe Asp Pro Pro Pro Phe Gln Glu Arg Asn Leu Ser Gly Gly Tyr
        115             120             125

Leu His Ile Tyr Glu Ser Gln Leu Cys Cys Gln Leu Lys Leu Trp Leu
    130             135             140

Pro Val Gly Cys Ala Ala Phe Val Val Val Leu Leu Phe Gly Cys Ile
145             150             155             160

Leu Ile Ile Trp Phe Ser Lys Lys Lys Tyr Gly Ser Ser Val His Asp
            165             170             175

Pro Asn Ser Glu Tyr Met Phe Met Ala Ala Val Asn Thr Asn Lys Lys
            180             185             190

Ser Arg Leu Ala Gly Val Thr Ser
        195             200


<210> 25
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<212> DNA
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<220>
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<400> 25
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<210> 26
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<213> Artificial Sequence

<220>
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     Oglionucleotide

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tggtgaccta ccacatccca cag                   .                 23


<210> 27
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
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     Oglionucleotide

<400> 27
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<210> 28
<211> 24
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<220>
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     Oglionucleotide

<400> 28
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<210> 29
<211> 28
<212> DNA
<213> Artificial Sequence

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     Oglionucleotide

<400> 29
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<210> 30
<211> 28
<212> DNA
<213> Artificial Sequence

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      Oglionucleotide

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cccaacgtgt actggatcaa taagacgg                              28


<210> 31
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Synthetic
      Oglionucleotide

<400> 31
gcgtgctgag gatcgcacgg acccccag                              28


<210> 32
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Synthetic
      Oglionucleotide

<400> 32
gcctctagaa agagctggga c                                     21


<210> 33
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
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cgccgtgttc catttatgag c                                     21


<210> 34
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<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Synthetic
      Oglionucleotide

<400> 34
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<210> 35
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<212> DNA
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<213> Artificial Sequence

<220>
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      Oglionucleotide

<400> 35
actattaggg tcatgcac                                          18